Invited Review: The Role of Cow, Pathogen, and Treatment Regimen in the Therapeutic Success of Bovine *Staphylococcus aureus* Mastitis

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ABSTRACT

Staphylococcus aureus is an important cause of udder infections in dairy herds. Both lactational and dry cow therapy are part of *Staph. aureus* control programs. Reported cure rates for *Staph. aureus* mastitis vary considerably. The probability of cure depends on cow, pathogen, and treatment factors. Cure rates decrease with increasing age of the cow, increasing somatic cell count, increasing duration of infection, increasing bacterial colony counts in milk before treatment, and increasing number of quarters infected. *Staphylococcus aureus* mastitis in hind quarters has a low cure rate compared with front quarters. Antimicrobial treatment of intramammary infections with penicillin-resistant *Staph. aureus* strains results in a lower cure rate for treatment with either β -lactam or non- β -lactam antibiotics. Other strain-specific factors may affect the probability of cure but routine diagnostic methods for use in bacteriology laboratories or veterinary practices are not yet available. The most important treatment factor affecting cure is treatment duration. Increased duration of treatment is associated with increased chance of cure. Economically, extended treatment is not always justified, even when indirect effects of treatment such as prevention of contagious transmission are taken into consideration. Usefulness of treatment trials could be improved by standardization of case definitions, consideration of host and strain factors, and sufficient statistical power. Treatment of young animals with penicillin-sensitive *Staph. aureus* infections is often justified based on bacteriological cure and economic outcome, whereas treatment of older animals, chronic infections, or penicillin-resistant isolates should be discouraged. **Key words:** *Staphylococcus aureus*, mastitis, treatment, cure

INTRODUCTION

The prevalence of IMI with *Staphylococcus aureus* can be reduced through implementation of the 5-point

program (Hillerton et al., 1995; Zadoks et al., 2002a). This program, developed in the 1960s (Neave et al., 1969) and later extended to the 10-point program (National Mastitis Council, 2001), covers effective udder health management practices for control of all mastitis pathogens. For contagious organisms such as most *Staph. aureus*, proper milking procedures, use of post-milking teat disinfectants, biosecurity to prevent introduction of pathogens, and segregation or culling of chronically infected animals are important aspects of these plans. Failure to control *Staph. aureus* mastitis may be caused by failure to implement the 10-point program correctly or completely (Barkema et al., 1998b).

For successful implementation of a mastitis control program, it is important to identify *Staph. aureus*-infected cows and heifers quickly, and deal with them in such a way that the opportunity for spread of the pathogen in the herd is reduced (Zadoks et al., 2002a). This can be done through segregation, culling, or treatment. Many herds do not have facilities or labor to handle additional groups or individual animals and are not willing to cull infected animals (Wilson et al., 1995; Sears, 2002). As a result, interest in treatment of *Staph. aureus* mastitis has reemerged in some countries in recent years as exemplified by numerous studies on treatment of nonlactating heifers and the availability of drugs specifically for treatment of subclinical mastitis during lactation (e.g., Borm et al., 2005; Deluyker et al., 2005). The interest in treatment may also be stimulated by lower regulatory limits for bulk milk SCC (BMSCC) or premium bonuses (Allore et al., 1998). Reported cure rates for *Staph. aureus* mastitis range considerably; for example, cure rates for subclinical *Staph. aureus* mastitis range from 4 to 92% (Schällibaum et al., 1981; Remmen et al., 1982; Ziv and Storper, 1985; Owens et al., 1988, 1997; Timms, 1995). Treatment regimens, and cow- and pathogen-related factors have a strong impact on the probability of cure (e.g., Sol et al., 1994, 1997, 2000; Wilson et al., 1999; Deluyker et al., 2005), but are often not included in the consideration to treat a cow with *Staph. aureus* mastitis. Additionally, estimates for cure rates may differ because of

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Table 1. Cow, pathogen, and treatment factors affecting cure in treatment trials of clinical or subclinical *Staphylococcus aureus* mastitis during lactation or the dry period¹

	Trial						
	Deluyker et al., 2005	Deluyker et al., 2005	Dingwell et al., 2003	Osteras et al., 1999	Sol et al., 1994	Sol et al., 1997	Sol et al., 2000
Lactation stage	Lactation	Lactation	Dry period	Dry period	Dry period	Lactation	Lactation
Clinical manifestation	Subclinical IMI	High SCC ²	NA	NA	NA	Subclinical IMI	Clinical IMI
Cow factors							
Parity	Decrease ^{3,S}	Decrease ^S	NS	Decrease ^S	Decrease ^S	Decrease ^S	Decrease ^N
DIM ⁴	Increase ^S	NS	NA	NA	NA	Increase ^S	Decrease ^N
Rear vs. front quarters	NR	Decrease ^S	Decrease ^S	Decrease ^S	Decrease ^S	Decrease ^S	NS
SCC	NR	Decrease ^S	Decrease ^S	Decrease ^S	Decrease ^S	Decrease ^S	Decrease ^N
No. of positive samples	NR	NR	Decrease ^S	NR	Decrease ^S	Decrease ^N	NR
No. of cfu	Decrease ^S	NS	Decrease ^S	NR	NR	NR	NR
No. of quarters	NR	NR	NS	Decrease ^S	Decrease ^S	NR	NR
Pathogen factor							
Penicillin-resistant	NR	NR	NR	Decrease ^S	NR	Decrease ^N	Decrease ^S
Treatment factor							
Duration	Increase ^S	Increase ^S	NA	NA	NA	NR	Increase ^S

¹NA = Not applicable; NS = not significant and numerical differences in cure rate = <10%; NR = Not reported.

²Results for high SCC are not specific to *Staph. aureus*, but did not differ between pathogen species (Deluyker et al., 2005). Quarter-level factors that were tested in models for *Staph. aureus* mastitis by Sol et al. (1994, 1997, 2000) were not included in infection model but in SCC model of Deluyker et al. (2005). SCC results from Deluyker et al. (2005) are included to allow for comparison between studies.

³Change in cure rate with increase in host, pathogen, or treatment level risk factor. Only statically significant ($P < 0.05$) and numerical differences with a difference in cure rate >10% are included. Statistical significant factors for cure are marked with an S, while numerical differences are marked with an N.

⁴Time frame for clinical mastitis different (all levels <100 DIM) than for subclinical mastitis (<100 DIM, 101–200 DIM, >200 DIM).

differences in study design, such as definition of cases and cure, and length of follow up.

In this article, we present an overview of cow, pathogen, and treatment factors that are associated with cure after treatment of *Staph. aureus* mastitis. Additionally, the design of clinical trials to evaluate treatment success is discussed to illuminate possible causes of discrepancies between existing reports and to suggest guidelines for future research. Finally, economic aspects of treatment of *Staph. aureus* mastitis are considered. Although not all *Staph. aureus* infections are responsive to treatment, the authors believe that use of existing information can result in more efficient and prudent use of antibiotics without compromising mastitis control or BMSCC levels.

HOST-LEVEL FACTORS

Cow- and quarter-level factors that affect the probability of cure of *Staph. aureus* IMI have been the subject of numerous studies (Sol et al., 1994, 1997, 2000; Osteras et al., 1999; Dingwell et al., 2003; Deluyker et al., 2005). Trials have been performed in cows with either clinical mastitis (Sol et al., 2000), subclinical IMI during lactation (Sol et al., 1997; Deluyker et al., 2005), or subclinical IMI at dry-off (Sol et al., 1994; Osteras et al., 1999; Dingwell et al., 2003). The similarity in results between those and other studies is striking (Table 1).

Higher parity is associated with a lower chance of cure (Ziv and Storper, 1985; Pyörälä and Pyörälä, 1998; Taponen et al., 2003b; Table 1). Higher SCC is also associated with a lower chance of cure (Owens et al., 1988; Table 1). Infections in hind quarters are less likely to be cured than those in front quarters (Table 1). Some factors were not evaluated in every study but showed a similar direction of effect in all studies in which they had been evaluated. A higher number of *Staph. aureus*-positive samples before treatment was associated with a decreased cure risk (Sol et al., 1994, 1997; Dingwell et al., 2003). Similarly, higher colony-forming unit counts in the milk sample were associated with a lower probability of cure (Dingwell et al., 2003; Deluyker et al., 2005). When multiple quarters of a cow are infected, cure rate is lower at the cow level (Sol et al., 1994; Osteras et al., 1999; Janosi et al., 2001) and at the quarter level (Sol et al., 1994).

As an example, Sol et al. (1997) evaluated the association between cow factors and the proportion of cure of subclinical *Staph. aureus* IMI in a meta-analysis of several clinical trials involving a variety of antimicrobial compounds. All quarters included in the meta-analysis were sampled 7 d before treatment, on the day of treatment, and at 16 and 30 d after treatment. Of the 143 quarters treated, 34% were still culture-negative for *Staph. aureus* 30 d after treatment. Based on the results of the regression model, a prediction could be

made of the probability of cure of a specific animal with a subclinical *Staph. aureus* IMI. For instance, the probability of cure of an older cow treated at 150 DIM, infected in a hind quarter with an SCC of 2,000,000 cells/mL is approximately 1%: $1/1\{1 + \exp[-1 \times (0.40 - 1.25 - 1.05 - 1.53 - 0.95)]\}$. Such a cow should not be considered a candidate for treatment but rather a candidate for segregation, culling, or cessation of lactation in the infected quarter (Middleton and Fox, 2001). By contrast, a heifer infected in a front quarter treated at 220 DIM with an SCC of 500,000 cells/mL had a 61% probability of cure: $1/1\{1 + \exp[-1 \times (0.40)]\}$. For such an animal, treatment may be a viable option, especially when one takes into consideration that the return on the investment of raising this heifer is yet to come. The range of cure probabilities covered by this prediction formula also largely covers the range of average cure probabilities reported in other studies. Thus, differences between studies may, in part, be the result of differences in selection criteria used to enroll animals. The knowledge that cow factors can be used to predict the probability of cure and could be used to select candidates for mastitis treatment as opposed to culling is vastly underused by pharmaceutical companies, farmers, and veterinarians.

The number of quarters infected with *Staph. aureus* is an important predictor of cow-level cure during the dry period, with more infected quarters resulting in a lower risk of cure at cow and quarter level (Sol et al., 1994; Osteras et al., 1999; Janosi et al., 2001; Table 1). In addition, if not all quarters of a cow are cured, the uninfected quarters of that animal are at higher risk of (re)infection with the pathogen, probably as a result of auto-reinfection; that is, reinfection of cured quarters by noncured quarters within the same cow (Zadoks et al., 2001). Quarter location was also a consistent factor, with hind quarters showing significantly lower cure rates. Generally, hind quarters have higher infection risks (Barkema et al., 1997, 1998a). One could speculate that the larger volume of hind quarters relative to front quarters makes it a factor affecting cure.

Duration of *Staph. aureus* IMI (often measured as the number of subsequent samplings positive for *Staph. aureus*) seems to have a significant effect on cure rate (Sol et al., 1994; 1997; Dingwell et al., 2003; Table 1). In a study that compared cure of *Staph. aureus* IMI with a <2 wk and ≥ 4 wk duration, bacteriologic cure rates were 70 and 35%, respectively (Owens et al., 1997). It should be noted, however, that in that study, the short IMI were all experimentally induced whereas the longer duration IMI had occurred naturally. Experimental infections were induced with a single penicillin-sensitive *Staph. aureus* strain. Strain types found in natural IMI were not described. Thus, evidence pre-

sented in that study (Owens et al., 1997) should be reviewed with these differences in mind. In on-farm situations, it is unlikely that subclinical IMI will be detected within 2 wk of occurrence.

In almost all studies that were reviewed, bacteriologic cure rate was higher if cow-level SCC was lower in the milk recordings before treatment (Owens et al., 1988; Table 1). In some of the studies, a threshold of 1,000,000 cells/mL was used to distinguish high and "low" SCC (Sol et al., 1997). High cow-level SCC may also indicate that multiple quarters within the udder are infected. Palpable changes in an udder quarter are a contraindication for treatment (Friton et al., 1998; Deluyker et al., 2005). Reasons for reduced cure rates with chronicity have been explored and will be summarized later (under Treatment Factors).

Increasing parity was associated with lower cure risk in virtually all studies (Table 1). Pyörälä and Pyörälä (1998) report 57% cure of heifers with clinical *Staph. aureus* mastitis and 27% cure of older animals when treated with penicillin G. For penicillin-sensitive isolates, Ziv and Storper (1985) report 80% cure at quarter level in heifers and 50% or less cure in older animals after 4-d treatment of subclinical mastitis. Taponen et al. (2003b) report 92 and 67% quarter level cure of heifers and older animals, respectively, for penicillin-sensitive isolates causing clinical mastitis. In general, older cows are more likely to become infected and clinically diseased (for example see Barkema et al., 1998a; Zadoks et al., 2001). A larger mammary gland size in older animals may contribute to reduced chances of cure, because the antibiotic must diffuse through a larger tissue volume, and a larger volume of tissue needs be cleared of infection. This argument is similar to the one made for number of infected quarters and for the difference between front and rear quarters, but we are not aware of scientific evidence supporting this hypothesis. There is some evidence that the prevalence of penicillin resistance among *Staph. aureus* isolates is higher in older cows. Sol et al. (2000) found 7 of 39 (18%) of isolates from animals in first or second lactation to be penicillin-resistant, whereas 49 of 119 (41%) isolates from older animals were penicillin-resistant. Penicillin resistance is associated with a lower chance of cure (see below). Thus, the apparent host or age effect might partially be explained by a pathogen effect; that is, penicillin resistance.

PATHOGEN FACTORS

Many different strains of *Staph. aureus* exist, as shown by a large variety of strain-typing methods (Aarestrup et al., 1995; Zadoks et al., 2002b; Smith et al., 2005). In most herds, one strain of *Staph. aureus*

predominates, due to the contagious nature of such strains, but the predominant strain usually coexists with a number of other *Staph. aureus* strains (Zadoks et al., 2000). In vivo, strains differ in their ability to spread within herds (Smith et al., 1998) and in their ability to cause SCC elevation, clinical mastitis, or persistent infections (Zadoks et al., 2000; Haveri et al., 2005b) or milk production losses (Middleton and Fox, 2001). In vitro, strains differ in their ability to withstand killing by neutrophils (Mullarky et al., 2001), form biofilms (Fox et al., 2005), or invade mammary epithelial cells (Hensen et al., 2000). For each of the traits studied in vitro, an effect in vivo can be postulated. Biofilm formation and invasion into mammary cells can be expected to protect *Staph. aureus* from the host immune response and from antibiotics by making the bacteria inaccessible (Lammers et al., 1999; Kerro Dogo et al., 2002; Vasudevan et al., 2003; Cucarella et al., 2004). The ability to survive phagocytosis by neutrophils would protect the bacteria even if they were exposed to the host immune response, except maybe in the case of antibiotics that penetrated intracellularly, such as macrolides (Janosi et al., 2001). Strain-specific characteristics can be expected to affect the probability of cure of *Staph. aureus* IMI, and studies to that effect are starting to emerge. Dingwell et al. (2004) observed a strain-specific response to dry cow treatment (DCT) with tilmicosin or cloxacillin. The predominant groups of strains, named A, D, and F, responded equally well to tilmicosin treatment (71, 75, and 80% cure, respectively), whereas a marked difference in response to cloxacillin treatment was observed (46, 87, and 33% cure, respectively). Luby and Middleton (2005) and Tikofsky and Zadoks (2005) noted that isolates that belonged to a variety of strains that occurred infrequently in a herd seemed to respond better to treatment than the predominant strain. Neither study, one using pirlimycin treatment with or without vaccination and one using homeopathic remedies, was powerful enough to yield statistically significant results.

Antimicrobial Resistance

An obvious reason for failure to cure in response to treatment is resistance of the infecting *Staph. aureus* strain to the antibiotic used for treatment. The choice of treatment should be based on knowledge of the antimicrobial sensitivity of the *Staph. aureus* strain. When treating subclinical infections, treatment can be postponed until results of cow-level sensitivity testing are available. For clinical mastitis, treatment choices can be based on herd-level knowledge of the sensitivity patterns of predominant strains. Such herd-level knowledge can be obtained through sensitivity testing of clinical

isolates after treatment has been initiated. Results from previous clinical cases can then be used to develop a herd-level treatment plan for subsequent clinical cases (Roberson, 2003). Some authors go as far as advocating sensitivity testing before treatment of mild clinical mastitis is initiated (Taponen et al., 2003b). Selection of antibiotics for treatment based on in vitro susceptibility to antibiotics is no guarantee for treatment success in vivo. According to one study, in vitro testing can be used as a predictor for cure for *Staph. aureus* infections of less than 2 wk duration, but not for chronic IMI (IMI of more than 4 wk; Owens et al., 1997). The low average probability of cure for chronic IMI (35%) and the low number of cows with chronic *Staph. aureus* IMI may have affected that study's power to detect an association between in vitro and in vivo results. Although the value of susceptibility testing for treatment of clinical mastitis is debated (Constable and Morin, 2003), some authors (Ziv and Storper, 1985; Taponen et al., 2003b; Pyörälä, 2005; this review) are of the opinion that sensitivity testing should precede treatment, certainly in the case of subclinical mastitis, and that choice of inappropriate drugs should not be an excuse for treatment failure when sensitivity testing is available.

Penicillin resistance is probably the most well known antibiotic resistance of *Staph. aureus*. The prevalence of penicillin resistance among *Staph. aureus* appears to have decreased in the United States in recent years (1994 to 2001; Erskine et al., 2002; Makovec and Ruegg, 2003); by contrast, an increase in penicillin resistance was reported in Finland (Myllys et al., 1998). Reported resistance levels differ considerably between countries, ranging from 20–30% for Denmark and Norway (De Oliveira et al., 2000), to more than 85% for small isolate collections from Ireland (De Oliveira et al., 2000) and Brazil (Costa et al., 2000). Even within countries, estimates of resistance prevalence may vary widely. For example, estimates of the prevalence of penicillin-resistance in bovine *Staph. aureus* from the United States range from just over 30% (Makovec and Ruegg, 2003) to more than 70% (De Oliveira et al., 2000). Differences between and within countries or studies may partly be due to differences in methodology. Methods used to measure penicillin resistance include agar disc-diffusion assays (Tikofsky et al., 2003), agar dilution testing, nitrocefin testing, and methods for detection of genes encoding penicillin resistance (Haveri et al., 2005a). So far, only one study comparing phenotypic and genotypic methods of resistance determination of *Staph. aureus* from bovine IMI has been published (Haveri et al., 2005a). According to this study, as much as 40% of genotypically resistant isolates may be classified as penicillin-susceptible by means of agar dilution testing

Table 2. Response of penicillin-sensitive and penicillin-resistant *Staphylococcus aureus* mastitis to lactational treatment

Manifestation	Penicillin-sensitive		Penicillin-resistant		Reference
	Compound	Cure ¹	Compound	Cure ¹	
Subclinical	Various	34% (21/61)	Various	24% (20/80)	Sol et al., 1997
Subclinical	Penicillin G	48.9 to 56.5%	Penicillin G	8.7 to 14.3%	Ziv and Storper, 1985
	Penethamate iodide	62.7 to 68.8%	Penethamate iodide	12.5 to 7.7%	
			Methicillin	24.4 to 32.4%	
			Tamethicillin	20.0 to 48.6%	
Clinical	Penicillin G	43% (43/99)	Spiramycin or enrofloxacin	20% (11/56)	Pyörälä and Pyörälä, 1998
Clinical	Various	59% (60/103)	Various	41% (23/56)	Sol et al., 2000
Clinical	Penicillin G + neomycin	75.6% (65/86)	Amoxicillin + clavulanic acid	29.2% (7/24)	Taponen et al., 2003b
	or Penicillin G	56.1% (23/41)	or Spiramycin	33.3% (5/15)	

¹Numbers in parentheses refer to number of cures out of number of cases treated. For Ziv and Storper (1985), the percentage of infections that was eliminated at quarter level is shown. Results cover 2-d treatment (first number) and 4-d treatment (second number). Between 13 and 45 quarters were enrolled per treatment (combination of duration and compound).

(Haveri et al., 2005a). Other classes of antibiotics that are commonly used for treatment of *Staph. aureus* mastitis include macrolides (e.g., erythromycin, spiramycin, tilimicosin) and lincosamides (e.g., pirlimycin). Reported resistance levels of *Staph. aureus* to macrolide antibiotics are much lower than for penicillin and range from 14 to 17% based on phenotypic testing (Erskin et al., 2004).

When non- β -lactam antibiotics are used for treatment, the probability of cure is still lower for β -lactamase-producing, penicillin-resistant *Staph. aureus* than for penicillin-sensitive *Staph. aureus*. This has been observed in trials of lactational treatment of subclinical (Ziv and Storper, 1985; Sol et al., 1997; Table 2) and clinical *Staph. aureus* mastitis (Pyörälä and Pyörälä, 1998; Sol et al., 2000; Taponen et al., 2003b). One caveat is that not all trials are true comparisons of the response rate of penicillin-sensitive and penicillin-resistant strains. For example, Pyörälä and Pyörälä (1998) and Taponen et al. (2003b) report higher cure rates for sensitive than for resistant isolates, but use different active compounds for the 2 categories of isolates. Penicillin-sensitive isolates are treated with penicillin, whereas penicillin-resistant isolates are treated with spiramycin or enrofloxacin (Pyörälä and Pyörälä, 1998), or with spiramycin or amoxicillin-clavulanic acid (Taponen et al., 2003b). Response of penicillin-sensitive strains to nonpenicillin antibiotics is not assessed. For practical purposes, it suffices to remember that penicillin-resistant strains are far less likely to respond to available treatments than are penicillin-sensitive strains.

The mechanism underlying the association between β -lactam resistance and poor response to non- β -lactam treatment is unknown (Pyörälä and Pyörälä, 1998; Taponen et al., 2003b). In our opinion, the most likely explanation is that genes encoding penicillin resistance

are located on pathogenicity islands (Ito et al., 2003; Gill et al., 2005). In addition to resistance genes, pathogenicity islands may harbor virulence genes; for example, genes encoding the production of biofilm (Ito et al., 2003; Ubeda et al., 2003; Holden et al., 2004). Penicillin resistance could thus be an indicator of the presence of pathogenicity islands that contain virulence factors other than the penicillin resistance itself that contribute to the ability of bacteria to survive antimicrobial treatment. The use of some antibiotics, specifically fluoroquinolones, can even promote the dissemination of pathogenicity island-encoded virulence genes in *Staph. aureus*, at least in vitro (Ubeda et al., 2005). In many dairy regions (e.g., North America and Europe), use of fluoroquinolones is not permitted for treatment of mastitis in dairy cattle, but clinical trials using fluoroquinolone for *Staph. aureus* mastitis treatment in Finland have been published (Pyörälä and Pyörälä, 1998).

The cost of susceptibility testing has been raised as a major drawback of its use (Constable and Morin, 2003). Considering the poor response of penicillin-resistant *Staph. aureus* to treatment in many studies and countries, one could consider all penicillin-resistant *Staph. aureus* infections ineligible for treatment (Pyörälä and Pyörälä, 1998; Taponen et al., 2003b; this review). Antimicrobial susceptibility testing of *Staph. aureus* could then be limited to testing of penicillin sensitivity. Testing for β -lactamase production or penicillin sensitivity should be included in practice as a routine method (Taponen et al., 2003b). This recommendation has been implemented in The Netherlands, where penicillin sensitivity of *Staph. aureus* isolates is determined routinely by the Animal Health Service laboratory that performs the majority of the country's milk bacteriology on a fee-for-service basis.

Figure 1. Strain typing of *Staphylococcus aureus* isolates by means of automated ribotyping revealed a dominant strain (DUP-4025) that responded poorly to treatment and nondominant strains (DUP-4062, DUP-4069) that were associated with cure in a small-scale treatment trial (Tikofsky and Zadoks, 2005).

Strain-Specific Cure

Until recently, most methods used for typing of *Staph. aureus* strains were so-called comparative methods. These methods allow for comparison of typing results within studies, but do not provide the level of standardization that would be necessary for use of typing methods across laboratories (Struelens et al., 1998). Strain typing methods that are DNA sequence-based, such as multilocus sequence typing (MLST), provide standardized results that can be compared across laboratories, often using databases that are accessible through the World Wide Web (Enright and Spratt, 1999). Both comparative typing (Zadoks et al., 2002b) and MLST (Smith et al., 2005) have been used to show that some strains are more likely than others to cause IMI. Specifically, teat skin strains could be differentiated from strains found in milk. Multilocus sequence typing showed that the majority of IMI are caused by a limited number of *Staph. aureus* strains that belong to a specific clonal complex. This clonal complex was predominant in all 3 countries studied (Chile, United Kingdom, and the United States), representing 3 continents (Smith et al., 2005). It is tempting to speculate that this clonal complex comprises host- and organ-adapted strains that spread easily and respond poorly to treatment. Preliminary results reported by Tikofsky and Zadoks (2005) and Luby and Middleton (2005) fit that hypothesis if one postulates that the dominant strains mentioned in those studies belong to clonal complex 97, whereas the variety of other strains does not (Figure 1).

Multilocus sequence typing revealed that Newbould strain 305 does not belong to the clonal complex of

mastitis causing strains (clonal complex 97; Smith et al., 2005). Hence, Newbould 305 is not representative of natural IMI with *Staph. aureus* and results from treatment trials based on experimental IMI with Newbould strain 305 (e.g., Owens et al., 1988) will likely not be representative of results obtained after treatment of natural IMI. Based on insight into the structure of the population of mastitis-causing *Staph. aureus* strains, more representative strains can be used for future treatment, vaccination, or infection studies. Representative strains should be selected from clonal complex 97 (Smith et al., 2005). Representational difference analysis or comparison of complete genomes between isolates that do and do not belong to clonal complex 97 or between penicillin-susceptible and penicillin-resistant isolates may identify genes that are specific to clonal complex 97 or penicillin-resistant isolates, potentially revealing new treatment or vaccine targets (Holden et al., 2004).

The availability of standardized typing methods opens up the possibility of detection of strain-specific cure probabilities in a standardized manner so that strain-typing results from multiple studies can be combined into one database. Knowledge of strain-specific virulence or cure characteristics could subsequently be used for diagnostic purposes; for example, to propose strain-specific treatment or management strategies. To make this possible, we will need to improve our understanding of pathogen factors associated with cure of *Staph. aureus* mastitis through characterization of isolates from treatment trials around the world using a library typing method such as MLST, possibly enhanced with determination of presence of specific viru-

lence characteristics. Considering the rapid developments in genomics and molecular microbiology, the authors expect both insight into strain characteristics and availability of molecular methodologies for diagnostic laboratories to increase dramatically in the years to come. The fact that the same *Staph. aureus* strains seem to play a role in mastitis worldwide implies that insight from a study on isolates from one region may well be applicable in other parts of the world too. On the other hand, the worldwide prevalence of one clonal complex of mastitis-causing strains may also imply that these strains are highly adapted to survival in the bovine host, and that treatment of the majority of *Staph. aureus* infections will continue to be a challenge, unless infections are identified and treated early after onset. To summarize, methods for detection of strain-specific cure are now available. Both the bacterial core genome, as characterized by MLST, and virulence or resistance genes located on mobile genetic elements such as pathogenicity islands can be studied. Multiple studies show an association between penicillin resistance and cure probability that cannot be explained by the penicillin resistance itself. Additional studies show or suggest strain-specific response to other antibiotic or nonantibiotic remedies. Thus, we have both the tools and the incentive to expand our knowledge of strain-specific factors associated with cure.

TREATMENT FACTORS

Staphylococcus aureus is susceptible to a variety of antibiotics in vitro. However, farmers often complain that in vivo cure rates are disappointing. Several factors including the ability of *Staph. aureus* to survive inside neutrophils (Yancey et al., 1991; Mullarky et al., 2001), to form small-colony variants or L-forms (Owens and Nickerson, 1989; Brouillette et al., 2004), to induce fibrosis and formation of microabscesses (Ziv and Storper, 1985; Sordillo et al., 1989; Erskine et al., 2003), and to invade into mammary epithelial cells (Lammers, 2000; Kerro Dogo et al., 2002) are potential contributors to the poor response of chronic *Staph. aureus* to antimicrobial treatment.

In attempts to improve the response to treatment, various classes of antimicrobial compounds, drug combinations, application routes, and treatment durations have been investigated. In addition, the usefulness of alternative and complementary remedies such as polypeptides, cytokines, immunomodulators, and vaccines is being explored. Examples of various treatment regimens are discussed below.

Antimicrobial Drugs

Many antimicrobial drugs have been used for mastitis treatment, including compounds that do not readily

penetrate the mammary gland; for example, sulfonamides, penicillins with the exception of penethamate iodide, aminoglycosides, and early-generation cephalosporins (Ziv and Storper, 1985; Sandholm, 1995; Erskine, 2000). Several studies show little difference in response to different therapeutics. For example, 5 different treatments using penicillin-novobiocin, penicillin-streptomycin, cephalirin, tilmycosin, or a cephalonium-based product were equally effective in eliminating *Staph. aureus* from prepartum heifers, with cure approximating 100% (Owens and Ray, 1996; Owens et al., 2001). For treatment of clinical mastitis caused by penicillin-resistant isolates, amoxicillin-clavulanic acid and spiramycin were equally ineffective with only 31% cure (Taponen et al., 2003b). For DCT of *Staph. aureus*, no significant difference in cure rate (average 47.7%) was observed between animals treated with cephalonium and those treated with cloxacillin (Shephard et al., 2004). In other studies, differences between active compounds are observed. For example, comparison of the second-generation cephalosporin cefuroxime with cloxacillin for treatment of clinical *Staph. aureus* mastitis favored use of cefuroxime (52.4% of 21 cases and 12.5% of 8 cases cured, respectively; Wright, 2003). In another treatment trial of clinical mastitis, use of lincomycin plus neomycin compared favorably with the use of ampicillin plus cloxacillin (41.2 vs. 15.4% bacteriological cure, respectively; Deluyker et al., 1999). When the same active compound is used, treatment efficacy can differ considerably between different formulations of that compound, as shown by Ziv and Storper (1985) for penicillin G, methicillin, and their esters. For example, penethamate hydriodide, a weak base ester of penicillin G, was associated with a higher probability of cure than its parent compound after 4-d treatment of subclinical mastitis (68.8 vs. 56.5% cure, respectively). In many studies, different drugs are associated with different treatment regimens or product formulations. In the comparison of lincomycin-neomycin vs. ampicillin-cloxacillin for example, one product was in aqueous solution whereas the other was an oil suspension (Deluyker et al., 1999). It is not always possible to differentiate between the effect of the active compound and the effect of the commercial product and its route or dose of administration. Additional examples will be discussed below.

Drug Combinations

Some combinations of drugs seem to have synergistic effects. Lohuis et al. (1995) found that penicillin and neomycin acted synergistically against *Staph. aureus* isolated from bovine mastitis cases. This synergy was observed more frequently in penicillinase-positive

strains compared with penicillinase-negative strains. This, according to Lohuis et al. (1995), is striking and probably due to impaired penicillinase synthesis, which results in an increased susceptibility of *Staph. aureus* to penicillin. Hensen (2000) found a synergism between penicillin and neomycin for *Staph. aureus* adhered to epithelial cells in vitro. Taponen et al. (2003a), however, found equal cure rates in clinical mastitis due to penicillin-susceptible, gram-positive agents using penicillin G alone or in combination with neomycin. In their opinion, aminoglycosides have been introduced into mastitis preparations without any clinical evidence of better efficacy, based purely on in vitro studies. Janosi et al. (2001) even argue that comparison of parenteral treatment with spiramycin and intramammary treatment with spiramycin and neomycin was really a comparison of parenteral vs. intramammary spiramycin treatment because "neomycin is unlikely to have contributed to cure of *Staph. aureus* infections due to the intraphagocytic location of the pathogen in vivo." Interestingly, the research group that says neomycin does not contribute to the efficacy of treatment of clinical mastitis (Taponen et al., 2003a) reports on cure of penicillin-sensitive *Staph. aureus* causing clinical mastitis in a different study (Taponen et al., 2003b). In that study, cure after combined parenteral and intramammary treatment with penicillin G is claimed to be superior to parenteral treatment alone, with 75.6 and 56.1% cure, respectively. The authors do not discuss the fact that the combined treatment included an extra active compound that was not included in the parenteral treatment alone: neomycin (Taponen et al., 2003b). From a methodological point of view, the higher cure rate observed among the group receiving combined treatment might just as well be attributed to the administration of neomycin as to the combined use of parenteral plus intramammary penicillin. Use of a combination of drugs often constitutes extra-label use. Justification of the decision to use such treatments, accurate recording of treatments, and avoidance of antimicrobial residues are very important when extra-label treatments are used.

Duration of Treatment

Longer treatment is generally associated with a higher probability of cure (Owens et al., 1988; Table 3). Extended therapy, for example 5- or 8-d treatment of mastitis, as opposed to the 2- or 3-d treatment that is customary for clinical mastitis, has been investigated in numerous studies (Table 3). Field trials with commercial antimicrobial products showed higher proportions of cure when using extended treatment, both for treatment of clinical and subclinical *Staph. aureus* mastitis (Owens et al., 1997; Sol et al., 2000; Gillespie et

al., 2002; Deluyker et al., 2005). In a small-scale study, Gillespie et al. (2002) found cure rates of 13, 31, and 83% after 2-, 5-, or 8-d lactational treatment of subclinical *Staph. aureus* mastitis with pirlimycin based on 15, 16, and 6 cows, respectively. In a large multinational study, Deluyker et al. (2005) observed cure rates for subclinical mastitis of 6, 56, and 86% for no treatment, 2-d, and 8-d treatment, based on 63, 146, and 53 infections, respectively. Extended treatment does not always result in success as shown by an Australian study of lactational treatment of subclinical mastitis. The probability of cure in animals that received 6-d treatment (3 intramammary treatments with 200 mg of cloxacillin at 48-h intervals, combined with 3 parenteral erythromycin treatments at 24-h intervals) did not differ from the probability of cure in animals that did not receive any treatment (Shephard et al., 2000). For clinical mastitis, most evidence points toward an increased chance of cure with a longer duration of treatment. In a meta-analysis of 4 clinical *Staph. aureus* mastitis treatment trials, Sol et al. (2000) observed that extended treatment was 2.3 times more likely than standard treatment to result in bacteriological cure. In the meta-analysis, any of 5 intramammary treatment regimens that consisted of 3 intramammary infusions at 12-h intervals was considered standard treatment. Extended treatment consisted of a continuation of treatment for an additional 48 h, starting 12 h after the last trial treatment. Extended treatment was used at the farmer's discretion if standard treatment results were not satisfactory according to the farmer (Sol et al., 2000). In another clinical mastitis treatment trial, longer treatment was associated with a nonsignificant but numerically higher chance of bacteriological cure: 5-d treatment and 3- to 4-d treatment were associated with 42 and 29% cure, respectively (Pyörälä and Pyörälä, 1998). Dry cow therapy using 4-d parenteral treatment with spiramycin was significantly more successful than 1-d DCT with the same product (48 and 14% cure, respectively; Janosi et al., 2001). Other DCT studies that suggest comparison of treatments of various durations actually compare various combinations and doses of active compounds (Osteras et al., 1999) and are thus not true comparisons of short-acting and long-acting DCT.

Benefits of extended therapy protocols, such as higher proportions of cure, resulting in decreasing SCC, less risk of transmission, and improved marketability of milk, must be weighed against several drawbacks, including the price of the antibiotic, loss of milk due to withdrawal, increased risk for residues in the milk, and the potential of infecting the cow through repeated infusions via the teat canal (Janosi et al., 2001; Poelarends et al., 2001; Gillespie et al., 2002; Swinkels et al., 2005). The incidence of new IMI with *Escherichia coli*

Table 3. Association between duration of treatment and probability of cure in treatment trials of *Staphylococcus aureus* mastitis

Manifestation	Compound	Short treatment		Extended treatment		Reference
		Days	Cure ¹	Days	Cure ¹	
Subclinical	Pirlimycin	2	3 to 56% (n = 146)	8	10 to 86% (n = 53)	Deluyker et al., 2005
Subclinical	Pirlimycin	2	13.3% (2/15)	8	83.3% (5/6)	Gillespie et al., 2002
Subclinical	Penicillin	2	48.9%	4	56.5%	Ziv and Storper, 1995 ²
	Penethamate		62.7%		68.8%	
	Methicillin		24.4%		32.4%	
	Tamethicillin		20.0%		48.6%	
Clinical	Various	3–4	29% (26/91)	5	42% (27/64)	Pyörälä and Pyörälä, 1998
Clinical	Various	2	48% (31/64)	4	74% (29/39)	Sol et al., 2000 ³
Dry cow treatment	Spiramycin	1	14% (2/14)	4	48% (12/25)	Janosi et al., 2001

¹If a range of cure rates is shown, that range represents results for the range of combinations of host factors in the particular study, e.g., parity, SCC level, and quarter position. The number of quarters enrolled in the study (n) is presented. If one cure rate is shown, this presents the average cure rate without consideration of host factors, and the number of quarters cured out of number treated is shown.

²Results for penicillin and penethamate iodide refer to penicillin-sensitive strains; results for methicillin and tamethicillin refer to penicillin-resistant strains.

³Results are shown for β -lactamase-negative isolates. For β -lactamase-positive isolates, cure was 38% (15/40) for 2-d treatment and 50% (8/16) for extended treatment.

or *Klebsiella* spp. increased considerably with an increasing duration of intramammary treatment (Gillespie et al., 2002). This phenomenon has also been reported for *Streptococcus agalactiae* “blitz” therapy (Loeffler et al., 1995; Edmondson, 1997). The loss of milk due to drug residues results in a poor cost-benefit ratio for most antibiotic therapies during lactation (Craven, 1987) and this effect is exacerbated when extended therapy is used (Swinkels et al., 2005).

Route of Application

Most often when antimicrobial treatment of (sub)clinical mastitis is implemented by farmers, only intramammary treatment is used. However, with *Staph. aureus* mastitis, inflammation of the udder tissue is involved and systemic treatment may have a beneficial effect on cure (Ziv and Storper, 1985; Owens et al., 1988). In a clinical trial that compared intramammary treatment with amoxicillin alone and a combination of systemic penicillin G and intramammary treatment with amoxicillin, cure rates of subclinical *Staph. aureus* mastitis were 51% for the combination therapy, approximately twice as high as in the quarters of cows that received intramammary treatment alone (Owens et al., 1988). More recently, combined parenteral Procaine penicillin G and intramammary treatment with penicillin G plus neomycin of clinical *Staph. aureus* was compared with parenteral penicillin only, and again combined treatment was found to be more effective (Taponen et al., 2003b). Both studies (Owens et al., 1988; Taponen et al., 2003b) describe comparisons that differ in route of administration as well as presence or absence of a second active compound. Therefore, the exact contribution of administration route to cure rates is

undetermined. Combined parenteral plus intramammary treatment is not always associated with higher cure rates than intramammary treatment alone. Combined 2-d parenteral treatment with penethamate iodide and intramammary treatment with cefacteril was not more effective than 2-d intramammary treatment with cefacteril alone in a German study, suggesting that there is no advantage to combination of compounds or routes of administration (Friton et al., 1998). More than 50% of *Staph. aureus* isolates in this study were penicillin-resistant, so for many cases that were enrolled in the study the use of penethamate iodide was not appropriate (Friton et al., 1998). Besides, average SCC in the group that received intramammary treatment only was below 1,000,000 cells/mL, whereas average SCC was above 1,000,000 cells/mL in other treatment groups (Friton et al., 1998). According to Sol et al. (1997), the probability of cure is higher for animals with SCC below 1,000,000 than for those with SCC above that threshold.

Some studies that purport to compare the efficacy of local and systemic antibiotic treatment (Sérieys et al., 2005) do not describe a comparison of different routes of administration, but rather different active compounds that happen to be administered via different routes (Pyörälä, 2005). When comparing parenteral and local treatment, it is important to ascertain that therapeutic concentrations are reached in the udder with both treatments (Pyörälä, 2005). The total amount of antibiotics used for treatment may be larger for parenteral treatment than for intramammary treatment (Hillerton and Kliem, 2002), which could affect the economic benefit or the risk of development of antimicrobial resistance. In a comparison of intramammary and intramuscular DCT of *Staph. aureus*-infected cows with spiramycin,

Janosi et al. (2001) concluded that intramammary treatment was preferable over parenteral treatment because treatment results were similar (40 and 48% cure, respectively, at cow level, and 37 and 30% at quarter level), whereas treatment costs were lower for intramammary treatment.

Prepartum Heifer Treatment

Over the years, many studies on prepartum antibiotic therapy of dairy heifers have been performed and published. Most studies originate from Louisiana and Tennessee, 2 states in the southern United States (Nickerson et al., 1995; Owens and Ray, 1996; Oliver et al., 1992, 2004; Owens et al., 2001). More recently, a multistate trial was conducted that included southern and northern states as well as a Canadian province (Borm et al., 2005) and trials have also been conducted in Europe (Sampimon and Sol, 2005). The majority of prepartum IMI in heifers are caused by coagulase-negative rather than coagulase-positive staphylococci, with *Staph. aureus* reported in 2% of quarters (Oliver et al., 1992; 2003), 8% of IMI (Oliver et al., 2004); 24 quarters of 42 heifers (Owens and Ray, 1996); 15.4% of quarters of 233 heifers (Owens et al., 2001); and 31% of heifers and 14.9% of quarters (Nickerson et al., 1995). Response of *Staph. aureus* IMI to prepartum antibiotic therapy is usually good in heifers. Nickerson et al. (1995) reported cure probabilities of 90% for treated heifers vs. 55% for untreated heifers. Owens et al. (2001) reported cure rates close to 100% on average for treated quarters and between 20 and 40% for untreated quarters. The cure rate did not depend on choice and formulation of active compound (Owens et al., 2001). Oliver et al. (2004) did not find a difference in cure rate between treated quarters and control quarters, or between quarters treated with pirlimycin hydrochloride or penicillin-novobiocin. The low number of infections in each treatment group (6, 5, and 5, respectively) limited the power of this study (Oliver et al., 2004). As a note of caution, although prepartum treatment of *Staph. aureus* mastitis in heifers can be highly effective, this effect has mostly been demonstrated in Louisiana, where the prepartum prevalence of *Staph. aureus* mastitis in heifers is unusually high (Nickerson et al., 1995). In the multistate study reported by Borm et al. (2005), 5 of 9 herds did not have enough major pathogen IMI in heifers to assess the efficacy of prepartum treatment. Thus, prepartum treatment of *Staph. aureus* mastitis in heifers should only be considered in herds with high prevalence of *Staph. aureus* mastitis; a blanket recommendation to use prepartum treatment cannot be made.

Alternative Treatments

Several nontraditional antimicrobial remedies have been tested as treatment of *Staph. aureus* mastitis. Interest in so-called alternative treatments is stimulated by the limited effectiveness of conventional antibiotics, concern about development of antimicrobial resistance because of use of antibiotics in the dairy industry, and the growth of the organic dairy sector that restricts or prohibits the use of antibiotics. In vitro, combining penicillin G with bovine lactoferrin increased the antibacterial effect against *Staph. aureus* (Diarra et al., 2002). Bacteriocins (e.g., nisin) are antimicrobial polypeptides ribosomally synthesized by bacteria. Nisin has been shown to inhibit in vitro growth of *Staph. aureus* (Broadbent et al., 1989). In vivo, the cure rate was significantly higher among *Staph. aureus*-infected cows receiving a 3-dose treatment with nisin than in untreated controls (28% of 18 cows and 0% of 32 cows cured, respectively; Coughlin et al., 2004).

In addition to bacteriocins, various immunostimulants have been tested. Ginseng appears to have immunostimulatory effects that could activate the innate immunity of cows and contribute to the cow's recovery from *Staph. aureus* mastitis (Hu et al., 2001). Protocols for lactational treatment of *Staph. aureus* mastitis based on use of immunostimulants and homeopathic nosodes have been published (Karreman, 2004), but they were not effective when tested in a controlled trial of chronic subclinical *Staph. aureus* mastitis (Tikofsky and Zadoks, 2005). A clinical trial on use of the immunomodulator beta-1,3-glucan for treatment of *Staph. aureus* mastitis during the dry period did not show a significant positive effect (Persson Waller et al., 2003). Cytokines also have immunomodulatory effects. Use of cytokines in immunotherapy of *Staph. aureus* mastitis is not effective as stand-alone therapy (Alluwaimi, 2004; Takahashi et al., 2005), but cytokines may improve the bactericidal effects of certain antibiotics (Alluwaimi, 2004). For some alternative and complementary compounds, field trials are underway but others are still a long way removed from proven efficacy and routine application in dairy practice.

Vaccination as Treatment

Recently, vaccines to prevent *Staph. aureus* IMI have become commercially available. A relatively new approach is the use of vaccines in combination with antibiotics to enhance results of lactational treatment of *Staph. aureus* IMI (Sears and Belschner, 1999; Timms et al., 2000; Sears and McCarthy, 2003; Luby and Middleton, 2005). Although the results are encouraging, none of these studies report a significant positive effect of vaccination on treatment results. In some cases, the

power of the study may have been a limiting factor; for example, when only 12 cows (respectively, 7 and 5 per treatment arm) were enrolled in the vaccination trial (Luby and Middleton, 2005). In a 22-cow trial in Korea, administration of an autogenous toxoid-bacterin to lactating cows with *Staph. aureus* mastitis resulted in 27% cure of quarters in the vaccinated group, which was significantly higher than the 5% cure observed in the control group. Vaccination also resulted in a significant decrease of SCC (Hwang et al., 2000). Vaccination was not combined with antimicrobial therapy in this treatment trial. Response to vaccination may be strain-specific, which could result in farm-specific or regional differences in vaccine efficacy (Guidry et al., 1998).

More research is needed before combining lactational therapy with vaccination can be advised in the treatment of subclinical *Staph. aureus* mastitis during lactation.

DESIGN OF CLINICAL TRIALS

When comparing treatments with regard to cure of *Staph. aureus* mastitis, well-designed clinical trials are essential. Design characteristics for clinical trials evaluating mastitis treatments have been summarized before (Thorburn, 1990; Schukken and Deluyker, 1995). These characteristics include proper formulation of the hypotheses to be tested, randomization of treatment allocation to avoid bias, double- or triple-blind design, formal sample size and power calculation, clinically relevant outcome variables such as SCC, milk yield, and culling, and a report of reasons for loss to follow up during the trial (St. Rose et al., 2003). In several articles, the issue of quarter- vs. cow- vs. herd-level analysis was also addressed. Subsequent papers have addressed the statistical methodology to deal with correlated binary data (Schukken et al., 2003a) that are often present in mastitis treatment trials.

In addition to these characteristics, a number of trial characteristics need specific attention in *Staph. aureus* IMI trials. These additional characteristics have to do with host and pathogen factors affecting cure risk, and with the contagious behavior of *Staph. aureus*. They include definition of relevant outcome variables, a precise description of strains involved in the study, and conscious accounting for contagiousness of *Staph. aureus*.

Relevant Outcome Variables

The goal of treating a cow with *Staph. aureus* mastitis is both bacteriological cure and clinical cure (in the case of clinical mastitis trial) or a decrease of SCC (in the case of a subclinical mastitis trial). Absence of clinical

signs defines clinical cure, and absence of SCC elevation can be used to define subclinical cure. Several thresholds have been proposed to distinguish between “normal” and “elevated” SCC. Based on a number of studies, a cut-off of 200,000 to 250,000 cells/mL is a good measure to distinguish between infected and uninfected quarters (reviewed in Schukken et al., 2003b). Because SCC usually drops shortly after treatment (see for example Luby and Middleton, 2005), this variable should be measured for a longer period, at least 30 d. The same is true for postpartum SCC. Barkema et al. (1999) observed that SCC decreased after calving in both infected and uninfected quarters. In infected quarters, SCC remained higher, but still dropped during 6 subsequent samples postpartum. Therefore, in DCT trials, SCC should be followed for at least 30 d after calving. If the reinfection rate is included as an outcome parameter, SCC should be monitored for a longer period. Another important reason for treating cows with subclinical *Staph. aureus* mastitis is to prevent these animals from being culled. Therefore, culling is an important outcome variable and should be evaluated for a considerable time after treatment (e.g., one lactation or one year). We are not aware of any studies including this outcome measure.

Side Effects

Part of any clinical trial should be the monitoring of possible side effects. Clinical mastitis after treatment is such a side effect. Several reports (Edmondson, 1997; Gillespie et al., 2002) illustrate the importance of monitoring this particular side effect of treating subclinical mastitis during lactation. Clinical mastitis incidence and SCC in the other quarters of the treated cows should be monitored. In a study of clinical mastitis, Van Eenennaam et al. (1995) reported that there was a difference between treatment groups in the risk of occurrence of a subsequent case of clinical mastitis in other quarters of the enrolled cow. Cows that received parenteral oxytocin treatment had a significantly higher incidence (28%) of a second case of mastitis than cows that received intramammary treatment with cephalosporin (10%) in the quarter that had clinical mastitis at enrollment. On the other hand, positive side effects, such as decreased SCC in adjacent quarters (Sérisys et al., 2005), may also go unnoticed if side effects are not monitored.

Diagnosis of IMI

The diagnosis of IMI depends on sample collection and handling strategies, culture methods, and interpretation criteria for culture results. The methods and cri-

teria for diagnosis of infection affect both the selection of candidates for inclusion in treatment trials, and the definition of cure. Samples can be collected pre- or post-milking (Sears et al., 1991; Godden et al., 2002). Collection time does not appear to affect culture results if samples are frozen before culture (Godden et al., 2002). Freezing, preincubation, and centrifugation as well as inoculum volume affect the sensitivity of culture-based detection of *Staph. aureus* in milk samples (Lam et al., 1996a; Zecconi et al., 1997; Godden et al., 2002; Sol et al., 2002). According to some studies, single milk samples are sufficient to diagnose *Staph. aureus* infections (Erskine and Eberhart, 1988; Buelow et al., 1996). However, some of these studies use 100- μ L inoculum volumes, resulting in higher sensitivity (Lam et al., 1996a) whereas other studies use 50- μ L (Deluyker et al., 2005) or 10- μ L volumes (Zadoks et al., 2002a; Dingwell et al., 2003). To increase sensitivity of detection of IMI, and to account for the fact that shedding of *Staph. aureus* may be intermittent (Sears et al., 1990), the diagnosis of IMI can be based on culture results of multiple consecutive samples (Lam et al., 1996b; Zadoks et al., 2002a). Intervals between duplicate samples vary between studies (Buelow et al., 1996; Lam et al., 1996b; Zadoks et al., 2002a). The number of colony-forming units that is detected in a milk sample and SCC or California Mastitis Test (CMT) score can also be taken into consideration in the diagnosis of IMI (Barkema et al., 1998a; Osteras et al., 1999; Zadoks et al., 2002a; Deluyker et al., 2005). It is beyond the scope of the current article to review all procedures used for sample collection, handling, culture, and interpretation. In an attempt to standardize methodology, guidelines for sample collection, culture methods, and interpretation of culture results have been published by the International Dairy Federation and the National Mastitis Council. Although there is much variation in criteria for diagnosis of infection, there appears to be broad agreement that multiple culture-negative samples need to be obtained after treatment to consider a quarter or a cow cured of *Staph. aureus* infection (Sol et al., 1994; Sol et al., 1997; Osteras et al., 1999; Dingwell et al., 2003; Deluyker et al., 2005) with very few exceptions (Sol et al., 2000).

Precise Description of Cows in the Study

Given the importance of cow and quarter factors affecting the cure risk (Table 1), it is of particular importance to clearly describe all cows in the study with regard to these parameters. A formal comparison of these factors between all treatment groups in the study is essential. The inclusion criteria for the trial may completely determine the range of cure risks observed

in the study. When only young animals with one infected quarter and a relatively short duration of IMI are included in the trial, cure rates above 50% may be expected. When another trial includes all infected animals in the study, much lower cure risks would be expected. The real efficacy of treatments in these trials may be equal, but the trial design would result in very different observed and reported cure rates.

Reinfection of Cured Quarters

Quarters that recover from infection have an increased susceptibility to reinfection (Zadoks et al., 2001, 2002a). Hence, treatment may result in short-term beneficial effects (bacteriological and clinical cure), but when a full evaluation of the efficacy and economics of a treatment program is the goal of the study, it is important to include long-term follow up. As for clinical cure, animals should be followed for a sufficiently long time to evaluate the occurrence of relapses after initial clinical or bacteriological cure. In a study on treatment efficacy of nonsevere clinical mastitis, the short-term benefit of treatment with cephalixin vs. oxytocin was considered small (Guterbock et al., 1993). However, when animals were followed after the initial clinical cure, the risk of relapse (defined as a subsequent case of clinical mastitis in the same quarter within 21 d of the initial treatment) was much higher in oxytocin-treated cows compared with cephalixin-treated animals (31 vs. 12%; Van Eenennaam et al., 1995).

Strains Involved

As presented above, strain variation of *Staph. aureus* does occur within and between farms. This has important consequences for comparison of cure risks among cows, farms, and trials. Even more, it may have important consequences for the very definition of cure. As shown by Luby and Middleton (2005), quarters may be infected with different strains before and after treatment. If this is the case, the quarter has experienced both a cure and a reinfection. For evaluation of the particular treatment, this should be coded as a cure. As mentioned above, for evaluation of treatment programs on a farm, this reinfection should be considered. It is therefore advisable to perform strain typing of all strains isolated from treatment efficacy trials.

Strain characteristics such as antimicrobial resistance may differ between infected animals or between trial sites. All strains in treatment efficacy trials should be documented with regard to known modifiers of cure risk (see also Pyörälä, 2005). Secondly, in treatment trials that use bacterial challenge followed by treatment (e.g., Owens et al., 1988, 1997), one strain of

Staph. aureus is used to infect all cows in the study. Strains used in experimental studies, such as Newbould 305 (Owens et al., 1988), may not be representative of strains causing natural infections (Smith et al., 2005), and use of one strain does not reflect the occurrence of multiple strains of *Staph. aureus* that is commonly observed in dairy herds (Smith et al., 1998; Zadoks et al., 2000). Experimental models would reflect the natural situation more closely if strains from the udder-adapted clonal complex of *Staph. aureus* were used as challenge organism. Using MLST, it is easy to identify such isolates (Smith et al., 2005). Another drawback of challenge-and-treatment trials is that we are far more likely to be confronted with requests for treatment of chronic cases in practice than with treatment of cases within 2 wk of infection, as used in challenge trials. In many countries, 3- or 4-weekly screening of individual cow SCC is used to detect putative infections. A cow is suspected of having an IMI if she has at least 2 high SCC records. This would take at least 3 to 4 wk or longer due to fluctuations in SCC, and would be followed by additional delay due to bacteriological culture and decision making regarding treatment.

Contagious Nature of *Staph. aureus*

Due to the contagious nature of *Staph. aureus* mastitis, treatment of IMI will have both a direct and an indirect effect (Zadoks et al., 2002a; Barlow et al., 2005). Cure of an infection results in a decrease in *Staph. aureus* prevalence due to the cure of a treated quarter (the direct effect). In addition, cure of an infected quarter leads to lower exposure of adjacent quarters in the cow or other animals in the herd, and therefore, to fewer auto-reinfections or new infections with *Staph. aureus* (the indirect effect). With an increasing risk of transmission of *Staph. aureus* infections in a herd, the importance of the indirect treatment effect increases. It may even become more important than the direct effect. This is illustrated in Figure 2, which shows the indirect effect of treatment relative to the direct effect of treatment. The direct effect is independent of the rate of transmission, whereas the indirect effect increases with increasing transmission. The transmission rate is represented as R , the reproduction ratio or the number of new infections caused by an existing infection during its infectious lifetime (Zadoks et al., 2002a).

The contagious nature of *Staph. aureus* should be taken into account when evaluating the effect of treatment of infected quarters and when evaluating treatment programs in herds. At the quarter level, the effect of treatment may be underestimated if contagious transmission results in reinfection of a cured quarter. In herds with high prevalence, infection pressure will

Figure 2. Simplified representation of the direct and indirect treatment effects. Treatment effect is measured as the reduction in the number of infected cows and it is graphed against the contagiousness of the organism. The graph represents a fictional 100-cow dairy with initially 5 *Staphylococcus aureus*-infected cows. A 6-mo window is presented in which 50% of the initially infected cows are treated with a 50% cure rate, and the cull risk for all animals is 20%. The actual cure of infected cows is the direct treatment effect. Contagiousness (represents Reproduction Ratio – R_0) is the number of new IMI in the 6-mo window per average *Staph. aureus*-infected cow present. Indirect treatment effect is the reduction in the number of newly infected cows that can be attributed to the cure of existing infections.

be high, resulting in a higher proportion of reinfections or apparent failures to cure. This is particularly true when so-called ‘Staph pens’ are used to house all cows that have ever been infected with *Staph. aureus*. In some situations, strain typing can help to distinguish cure followed by reinfection with a different strain from noncure. However, strain typing cannot distinguish between noncure and cure followed by reinfection with the same strain. The predominance of a contagious strain of *Staph. aureus* in most herds may make the differentiation between cure and noncure impossible. Statistical methods whereby prevalence is used as a covariate may be used to account for exposure to other infected cows. However, this may not be feasible in all studies. The minimum would be to report herd prevalence of *Staph. aureus* and the housing and management characteristics of the treated cows during the full period of the trial. The effect of treatment may also be underestimated at the herd level, if prevention of infections in herd mates is not taken into consideration as one of the positive effects of treatment (Swinkels et al., 2005).

ECONOMICS OF TREATMENT

Success of treatment is often measured in terms of clinical cure, bacteriological cure, and SCC or linear

Table 4. Herd, treatment, pathogen, and host factors influencing the economic outcome of lactational treatment of *Staphylococcus aureus* mastitis under Dutch conditions (Swinkels et al., 2005)

Treatment	Pathogen	Host ¹	Cure (%)	Net benefit (€)	
				Low transmission (R = 0.32) ²	High transmission (R = 5.3) ²
Short (3-d)	Penicillin-sensitive	Young, low SCC, late lactation	59.9	+5	+205
		Old, high SCC, midlactation	5.5	-52	-34
	Penicillin-resistant	Young, low SCC, late lactation	47.8	-8	+152
		Old, high SCC, midlactation	3.4	-54	-43
Long (8-d)	Penicillin-sensitive	Young, low SCC, late lactation	86.1	-30	+257
		Old, high SCC, midlactation	19.3	-100	-36
	Penicillin-resistant	Young, low SCC, late lactation	79.0	-37	+226
		Old, low SCC, early lactation	16.5	-103	-48

¹Parity: young = first or second parity; old = third or higher parity; SCC: low = lnSCC <6.9; high = lnSCC 6.9 or higher; LS = lactation stage: early <100 DIM; mid 100 < DIM ≤ 200; late >200 DIM.

²R = reproduction ratio = average number of new infections caused by an infectious individual during its infectious lifetime. R depends on strain and management.

score cure. Dairy farming is an economic enterprise, and it could be argued that the real measure of cure should be “economic cure”; that is, a treatment outcome that offsets the costs of treatment. Regulatory and economic conditions vary widely between countries. Differences exist in regulatory limits for BMSCC, milk and component pricing systems, quality premium payments and penalties, availability of antibiotics labeled for mastitis treatment or prevention, use and cost of hired labor, quota systems, and so on. As a result, an economic analysis performed for conditions in one country may not be applicable in another country. Some general considerations with regard to economic analysis will be outlined below, with examples drawn from countries with and without quota systems and with various legal limits for BMSCC.

Early reviews of the efficacy and financial value of antibiotic treatment of mastitis focused on cow-level cost-benefit analysis (Craven, 1987; De Graves and Farrow, 1993). In these analyses, which were not specific for *Staph. aureus*, lactational treatment of clinical mastitis was considered economically justified, but lactational treatment of subclinical mastitis was not. The probability of cure has a large impact on the economic benefit of treatment (Table 4). For example, both cure probability and economic benefit are high for *Streptococcus agalactiae* mastitis (Yamagata et al., 1987; Edmondson, 1989). The chance of cure is much lower for *Staph. aureus* than for *Strep. agalactiae*, which has led to a prevailing perception that treatment is not economically justified, especially in the case of subclinical mastitis during lactation. However, as pointed out in the section on cow-level factors, the probability of cure can range from less than 5% to more than 80%. As a result, the economic benefit of lactational treatment of subclinical mastitis varies widely between animals

(Swinkels et al., 2005). For example, treatment of young animals with moderate SCC elevation and IMI with penicillin-sensitive *Staph. aureus* strains is economically viable but treatment of older animals with strongly elevated SCC and IMI with penicillin-resistant *Staph. aureus* is not (Table 4). In addition to the chance of cure, the future value or retention pay-off of cows should be considered when making treatment decisions (Houben et al., 1994; Swinkels et al., 2005). Increased duration of treatment improves the chance of cure of *Staph. aureus* mastitis (Deluyker et al., 2005), but the increase in cure is not necessarily sufficient to offset the extra cost of antibiotics and discarded milk (Craven, 1987; Swinkels et al., 2005; Table 4).

Contagious transmission can play an important role in the epidemiology of *Staph. aureus*. This has implications for the design and interpretation of treatment trials, and for the economic benefit of treatment. The effect of treatment of a *Staph. aureus*-infected animal goes beyond the individual. An infected animal exposes noninfected herd mates to *Staph. aureus*, putting them at risk for new IMI. Successful treatment of an individual cow prevents IMI, clinical mastitis, SCC elevation, and milk production losses in other cows. Indirect or herd-level effects can be included in the cow-level cost-benefit analysis of *Staph. aureus* treatment, and have a strong impact on economic results (Table 4). In cost-benefit analyses based on partial budgeting, direct costs (the costs of antibiotics) and indirect costs (the cost of discarded milk) are weighed against direct benefits (increase in milk production) and indirect benefits (prevention of clinical flare-ups or prevention of transmission to other animals in the herd). The higher the risk of transmission, the higher the indirect benefit of prevention of transmission through cure of an infected quarter or animal. Partial budget model calculations

predict that 3-d lactational treatment of subclinical *Staph. aureus* mastitis (i.e., treatment with relatively low direct and indirect costs) would be profitable in herds with very low transmission (i.e., relatively low indirect benefits) only if the chance of cure were higher than approximately 55%. In herds with very high transmission, for example, during an outbreak of *Staph. aureus* mastitis, the indirect benefit of treatment is high, and 3-d and 8-d treatment were profitable at predicted cure probabilities as low as 13 and 28%, respectively (Swinkels et al., 2005).

Many economic analyses do not consider lactational treatment of *Staph. aureus* mastitis. Goodger and Ferguson (1987) showed that intervention was economically profitable in the control of a *Staph. aureus* mastitis outbreak in a large case herd, but the interventions did not include treatment. Dry cow therapy of *Staph. aureus* mastitis is usually economically profitable according to model calculations (Zepeda et al., 1998), but Zepeda et al. (1998) did not consider lactation therapy. The value of lactation therapy of clinical mastitis was analyzed by Allore and coworkers (Allore and Erb, 1998; Allore et al., 1998), but their model does not distinguish between treatment of *Staph. aureus* mastitis or mastitis caused by other pathogens. Seegers et al. (2004) argued, in a simulation study, that milk price differential due to BMSCC premiums of 1.5% of the milk price were essential to justify control programs that included lactational therapy of subclinical IMI, but their model is not specific for *Staph. aureus*.

In deterministic models, such as the ones used by Swinkels et al. (2005) or Zepeda et al. (1998), conclusions are based on the average outcome of alternative intervention strategies. Stochastic models, such as those used by Allore et al. (1998), allow for study of a range of possible outcomes, including unusual outcomes or "outliers." Outliers are by definition uncommon, but their impact may be of sufficient importance to warrant study of their likelihood and their economic impact. In the case of treatment of *Staph. aureus* mastitis, "outlier scenarios" can refer to bulk tanks exceeding regulatory limits for BMSCC or containing growth inhibitors, both of which are associated with penalties (Allore et al., 1998), or to unusual but potentially devastating mastitis outbreaks. As shown in experimental (Lam et al., 1996b) and observational studies (Smith et al., 1998), *Staph. aureus* outbreaks do occur. Although the likelihood of such outbreaks may be small, their impact can be very large. A risk analysis approach would be needed to incorporate the risk of outbreaks into herd or cow-level decision-making on treatment of *Staph. aureus* mastitis. Where identification of strain-specific risk factors for cure can be seen as the next frontier in pathogen-specific treatment decisions, a risk analysis ap-

proach may be the next frontier in economic justification of treatment decisions.

CONCLUSIONS

Many reports on treatment trials of *Staph. aureus* mastitis do not contain detailed descriptions of host factors of the treated animals, or of the strains causing the infections that are treated. Given that these factors highly influence cure after treatment, it is currently impossible to generalize and compare the results of these studies. For this reason, clinically relevant cow-level factors such as parity, SCC before treatment, duration of high SCC, and quarter location should be reported in future studies. Similarly, strains should be described using phenotypic or genotypic characteristics, preferably using standardized methods. We believe that conscious selection of cows that have a reasonable probability of cure after treatment could contribute to a major improvement in management and cure of *Staph. aureus* mastitis. In the authors' opinion, this process should consist of 1) early detection; for example, through monthly screening of cow-level SCC, followed by identification of infected quarters using the CMT and culture of milk samples from quarters with positive CMT results; 2) rapid follow up on information from tests; that is, CMT and culture immediately following receipt of SCC results by mail or e-mail, low turnaround time of milk samples, and prompt communication of results to herd managers; 3) access to cow-level data on parity, lactation stage, pregnancy status, production level, and mastitis and SCC history; 4) characterization of the *Staph. aureus* isolates with respect to penicillin sensitivity; 5) a protocol for decision making on the choice and duration of treatment. For example, a cow with a low chance of cure (e.g., less than 30%, or some other threshold that can be implemented in the decision process independent of the person following the decision-making protocol) is segregated and eventually culled, whereas a cow with higher chance of cure is treated. Clearly, cow factors such as expected milk production and strain factors such as antimicrobial resistance should play a role in the decision-making algorithm, and herd level or external factors such as within-herd prevalence, contagiousness, and price of milk, cull cows, and replacement heifers must also be weighed. This process should be continuous, with consistent monitoring of cow SCC and follow up on newly detected and treated cows, and on fresh cows or heifers and purchased animals. The greater the age of the cow, the number of infected quarters, the duration of infection, or the SCC level before treatment, the lower the chance of cure. When selecting candidates for treatment, duration of infection is key, and one of the best chances to

improve treatment results is through early detection of candidates for treatment. Because of the contagious nature of *Staph. aureus* mastitis, cure of subclinically infected animals will also result in reduced incidence and prevalence of *Staph. aureus* in the herd. Treatment factors such as duration of therapy will also affect the success of the program. Treatment of infections with penicillin-resistant strains is rarely successful or profitable.

Future development and research should be focused in a number of areas: a) better understanding of pathogen factors that affect cure, eventually resulting in strain specific treatment recommendations; b) algorithms for decision making that can be built into management programs or handheld devices, and that include economic, cow, and pathogen factors; c) evaluation of the practicality and economic viability of the use of decision-making algorithms; and d) further improvement of treatment regimens, possibly including vaccines and compounds other than antibiotics, evaluated in well-designed clinical trials and field trials. Special design characteristics of these *Staph. aureus* treatment trials have been described in this manuscript.

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