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Review Article

Evaluation of Three Methods for Determining whether an Antibiotic Medicine is Needed for the Treatment of Mild or Moderate Clinical Mastitis

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Introduction

In Denmark, dairy farmers get to decide for themselves if a cow with Clinical Mastitis (CM) should receive antibiotics. However, it is mandatory to collect a quarter milk sample for diagnostic purpose. This sample is tested later to confirm the treatment was justified, and use proactively to implement targeted management change to reduce the new infection risk. Farmers ship the samples to a laboratory within a few days. Most analyses are performed by the veterinary practices that oversee the health of the dairy farms, which means feedback takes several days. For mild or moderate cases of CM, antibiotic treatment is only beneficial for Intramammary Infections (IMIs) caused by specific organisms, including Staphylococcus aureus, Aggressive Non-aureus Staphylococci (NAS), Streptococcus species such as Str. uberis, Str. dysgalactiae, and Str. agalactiae, and streptococci-like bacteria. Finding a way to diagnose on the farm would make the whole process easier, save time on decisions, and cut down on unnecessary antibiotic use. Unfortunately, dairy farms may be considered low-resource settings and require tests that meet the ASSURED criteria defined by the W.H.O. [1]: Accuracy, Sensitivity, Specificity, User-friendliness, Rapid and Robust, Equipment-free, and Deliverable. The goal of this study was to evaluate three methods that could provide a quick, on-farm answer to the question: "Will antibiotic treatment benefit this cow?"

Material and Methods

For this study, we enrolled 12 dairy herds, ranging in size from approximately 250 to 1,300 animals. These herds had a herd health contract with a local veterinary clinic. Each clinic ran its own milk bacteriology program using selective media, with minor methodological differences between locations. Farmers were instructed to collect two CM milk samples: one fresh sample, which was refrigerated until use, and another sample preserved with 10% glycerol and immediately frozen. The fresh sample was initially tested on-farm using two already marketed rapid tests that have been assessed by others (Test-1 [2], Test-2 [3]). Both tests have been were prepared according to the manufacturers' instructions and placed in an egg incubator for 24 hours, at which point the results were read. Tests yielded three possible results: no growth (code 0), Gram-negative bacteria (-), or Gram-positive (+). Otherwise, the sample was declared contaminated (X). Both samples were then routinely shipped to the veterinary clinic. There, the veterinarians re-tested the fresh milk with the same commercial tests, and cultured milk using their routine methods (selective media). Finally, the frozen samples were shipped to a professional testing laboratory (Labeo, France) for culturing (on blood agar and in broth) and identification (MALDI-TOF). Samples in which more than three colony types were detected were declared contaminated. For vet clinics and the reference laboratory, final results were grouped into nine categories: STErile, MIXed, CONTaminated, S. aureus (SAU), S. uberis (SUB), Non-aureus Staphylococci (NAS), Other Gram-Positive (OGP), Coliforms, and Other Gram-Negative (OGN).

Results

To answer our initial question, 145 cows out of 183 deserved antimicrobial treatment (79.2%) (Table 1) according to the reference laboratory, with a possible enrollment bias. Out of 66 milk cultures results that have been captured, veterinary clinicians were able to correctly classify 51.5% of samples. However, based on in-clinic milk culture results, they correctly justify the antibiotic treatment in 88.5% of CM cases. Rapid tests, performed either at the veterinary clinic or on the farm, both yielded poor results, being able to identify CM cases likely to benefit from antibiotic therapy in only 36.1-61.5% of cases. Repeatability of the rapid tests is also concerning with a poor agreement between tests carried out on farm, and those performed in the veterinary practices (62.5 and 34,4% for test 1 and test 2, respectively).

Table 1: Results of milk cultures in the professional laboratory (top of the table), in-clinic milk cultures, and two rapid tests performed either at the vet clinic or on farm. Light gray areas represent results that should trigger an antibiotic treatment according to lab results, or according to the selected tests, with proportion of animals actually treated.

		Reference Laboratory (n=183)									
		STE	MIX	CONT	SAU	SUB	NAS	OGP	COL	OGN	
		9	11	15	8	57	25	29	27	2	
		9	145						29		
In-clinic milk culture (n=66)	STE	1							1	1	
	MIX			2		2				1	46/52
	CONT			1			1	1			
	SAU			1	1		1	4			
	SUB		1	3		18	2	1			
	NAS						2				
	OGP		1		1	1	1	1			
	COL		1	2		1	1	1	10		
	OGN										
Test-1 (vet testing, n=153)	0	6	1	6	1	3	6	9	7	1	
	-		2	2	2	23	4	2	9		
	+		6	5	4	26	7	12	5	1	63/124
	X		2			0	1				
Test-2 (vet testing, n=147)	0	5	1	6	2	6	5	9	4	1	
	-		7	4		26	6	4	12	1	
	+	1	1	3	4	15	4	7	1		43/119
	X	1	2		1	3	2	1	2		
Test-1 (farm testing, n=140)	0	6	1	3	1	3	7	4	4		
	-		3	4	2	11	5	6	10	1	
	+	1	5	6	5	28	5	9	6		62/112
	X					3	1				
Test-2 (farm testing, n=132)	0	5		3		2	6	3	3		
	-	1	4	3	2	10	3	4	13		
	+	1	3	5	4	20	6	10	2		64/104
	X		1	3	1	6	2	3	2	1	

Discussion

In this study, the prevalence of CMs likely to benefit from antibiotic treatment was high. The producers included clinical cases in the study that they believed required antibiotic treatment, and they were wrong in only 20.8% of cases. Even with pathogen identification disagreements between the reference laboratory and in-clinic labs, veterinarians were still able to satisfactorily identify animals requiring antibiotic treatment, improving producers' decision by barely 10 points. Within the context of a high prevalence of presumed necessary treatments, the Positive Predictive Value (PPV) reached 95.6%, while the Negative Predictive Value (NPV) remained acceptable at 72.2%. The performance of the two rapid on-farm tests was poor overall. While the PPVs for these tests when performed by veterinarians were satisfactory (ranging from 86.9% to 92.3%), NPVs were notably low, between 26.9% and 35.5%. These results contrast with those published elsewhere [2,3]. The low sensitivity of commercial test kits may be due to the very small volume of milk processed by these devices, much less than the 10 to 50 µL plated on the culture media. As a consequence, positive likelihood ratios (LR+) are high and the probability that an animal requires a treatment when the test is positive (+ or X) is higher than the prevalence. Conversely, the negative likelihood ratio (LR-) is very low, and a negative result (0 or -) is not informative for the veterinarian.

Conclusion

The two on-farm commercial test kits presented in this paper may certainly help identify CM cases eligible to antibiotic treatment. However, using the same tests to exclude negative-testing animals from antibiotic treatment would be hazardous, as it would significantly reduce their chance of recovery. Ultimately, while each test provided some diagnostic information, none met the ASSURED criteria. Improving antibiotic stewardship through more precise diagnosis may require exploring milk culturing performed by better-trained veterinary practitioners.

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