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Review Article

Molecular biological tools applied for identification of mastitis causing pathogens

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ABSTRACT

The molecular diagnostic tools became the gold standard of mastitis diagnosis in the last few years. They enable rapid, qualitative, quantitative and large scale diagnosis. In addition to their role in diagnosis, they can identify pathogens at the subspecies level which is necessary for the epidemiological studies. They are increasingly used in mastitis control programs through identification of suitable candidates for vaccine production and through the selection of mastitis resistant cattle breeds. The present molecular techniques are continuously improved and new techniques are developed in order to provide higher sensitivity and specificity and to minimize the costs. The present work aims to provide an overview of the modern molecular tools, discuss why they replaced the traditional tools and became the new gold standard in mastitis diagnosis through comparing both traditional and the molecular tools, explore the prospective of the molecular diagnostic techniques in mastitis diagnosis and control and to explore new horizons of using molecular assays in near future.

Contents

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1. Introduction

Mastitis is the most costly problem in dairy industry. The resulting economic losses include direct losses as (1) temporary/permanent decrease in milk production, (2) reduction of milk grade in subclinical cases due to the increased number of somatic cells and (3) the fully rejection of milk in clinical mastitic cases or due to antibiotic residues. In addition, indirect losses are also included as (1) premature culling of dairy cows and early replacement cost, (2) low cow sale price, (3) additional veterinarian and medication costs, and finally (4) the diagnostics/laboratory expenses [1,2].

Mastitis is the inflammation of the udder tissue which could be prompted by various infectious agents. It is characterized by the elevation of somatic cell count, and usually accompanied with physical, chemical and/or microbial changes of the milk. Mastitis pathogens are usually bacteria, however, molds, yeast, and prototheca may also induce mastitis. It is important to identify the mastitis prompting organism in order to (1) properly treat and select the suitable antibiotics, (2) understand their route of spread and evaluate the contagiousness of the case, (3) calculate their public health impact, (4) to judge the prognosis of the affected quarter/cow considering early culling decision, (5) select the suitable hygienic and preventive measures, and finally for (6) choosing the proper mastitis vaccine programs to be applied in the farm [2,3].

Field diagnosis of clinical mastitis is usually based on udder examination, changes in the physical properties of the milk and the increase in somatic cell count and even the use of ultrasonography [3,4] while the diagnosis of subclinical mastitis is more difficult and depends on indirect techniques such as California Mastitis Test (CMT), electrical conductivity, or the detection of body enzymes released due to tissue damage (e.g. LDH, and NAGase) [2].

An ideal diagnostic test must be sensitive, specific, rapid, repeatable and economic. Most conservative laboratories worldwide still consider bacterial isolation and cultivation to be the (Gold Standard) for the diagnosis of mastitis. The question if the culture still the gold standard and whether the PCR replaced it and became the modern gold standard is debated because both techniques have their strength aspects and weakness points (Table 1; [5,6]).

2. Major mastitis causing pathogens

More than 150 different bacterial species and subspecies are involved in the induction of bovine mastitis [7], out of these; only 10 groups are responsible for 95% of the recorded cases worldwide [2]. These pathogens classified as environmental or contagious pathogens depending on their primary reservoir and mode of

Table 1
Comparison between the old and the new gold standards: Bacteriological assays versus PCR showing the strength and weakness points of both techniques.

	PCR	Culture	Refs.
Technicality and costs	It is more expensive and requires special infrastructure and well trained skillful persons.	Standard media used for primary screening are always available in most laboratories. However, they are not suitable for isolation of some pathogens such as Mycoplasma or *Mycobacterium bovis*.	[2,14,50]
Bias	PCR inhibitors present in mastitic milk, improper extraction or purification of the DNA from the sample may lead to false negative results. The use of column purification is recommended, however, if the mastitic milk is clotted, the purification process may be inefficient. The use of internal controls can differentiate between truly - and false negative results. False positive results can occur due to nonspecific amplification if less restrictive PCR conditions are applied or if the primer selected is not specific enough. False positives due to DNA carryover effect and from contamination or teat canal colonization may also occur.	About 30% of milk samples taken from clinical and subclinical cases revealed negative bacterial growth after 48 h of incubation due to the death of the causative agent during transport/sample storage, the use of unsuitable culturing media or due to the presence of antibiotic residue or preservatives in the sample which inhibit the bacterial growth but not their molecular detection. Also due to overgrowth of contaminant microbes during sample transportation which may mask the real mastitis inducing microbes.	[5,32,73–76]
Public hazards	The use of Ethidium Bromide is a serious source of environmental contamination and public hazards.	The enrichment of the pathogens may lead to biological contamination/public health hazards can lead to laboratories acquired infections.	[77]
Screening capacity	Faster and adapted for screening purposes with lower costs per detected agent. The PCR can only detect the target pathogens according to the used However, the use of multiplex PCR overcomes this disadvantage	Has a broad spectrum screening capacity if the milk sample is cultured on blood agar a 37 °C for 48 h. Time consuming (24–48 h) and laborious especially if slow-growing bacteria are suspected.	[78]
Sensitivity and specificity	Higher sensitivity and specificity values due to its ability to detect both viable and killed organisms. PCR usually requires a small amount of target DNA and therefore has a higher detection limit.	The culturing process is not easily inhibited compared to PCR which can be inhibited by a wide range of PCR inhibitors present in mastitic milk such as proteinases, calcium ions, lactoferrin (leukocytes) and heme (in bloody milk), or due to programing mistakes of the thermocyclers.	[79,80]
Accuracy and repeatability	High detection level. The RT-PCR enables pathogen quantitation.	Culture enables multiplication of pathogens if present at low concentration and reflects the true active intramammary infections unlike PCR, because it detects only viable bacterial cells.	[29,30]
Typing	Differentiates among different genotypes of the same species and deliver antibiotic resistance profiles, which enables rapid treatment of mastitic cows.	Serotyping is not efficient enough. The antibiotic resistance test can be done but it is laborious and time consuming.	[8]
Others	The results delivered by PCR are in the form of digital data, which can be easily exchanged or stored. It is easier to store the PCR product (in refrigerator or freezer) for long periods, than storing cultured petri dishes, which will dry or will be masked by fungal growth if not properly preserved. Lyophilization of the culture or freezing in glycerin may offer good solutions for culture storage.	The source of the material (the grown cultures) remains available for test repetition/confirmation or for further investigations if needed in opposite to PCR when applied directly on the sample not on a culture. The source of the investigated material can multiply (as subculture) if the material was nearly exhausted or needed in large amounts, in opposite to extracted DNA in case of PCR.	[32,75,81,82]

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