Methicillin-resistant *Staphylococcus aureus* (MRSA) Prevalence in Mastitic Dairy Cows from San Bernardino County

Elizabeth Kurzawinski, Animal Science
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Mentor: Dr. Shelton Murinda

Abstract

The prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy cows with mastitis can result in major economic losses to the dairy industry, and is a potential health risk to humans who consume raw milk and milk products manufactured with raw milk. Few studies have been conducted determining the prevalence of mastitis in dairy cows caused by MRSA, in the United States. The objective of this study is to determine the prevalence of MRSA in dairy cows with mastitis from Chino and Ontario, California. Bacteria were isolated from 318 milk samples collected from individual cows were isolated. There are currently 71 presumptive *Staph. aureus* isolates (22%) to be confirmed by use of polymerase chain reaction (PCR) to target amplification of the *mec*A, *nuc, coa, mup* and *Staphylococcus* genus specific 16s rRNA genes. Antibiotic resistant profiles including the antibiotics cefoxitin, oxacillin, methicillin, and mupirocin will be determined for each isolate. Preliminary results suggest moderately low prevalence of *Staph. aureus*. If a low prevalence of MRSA in milk samples from dairy cows should be confirmed, veterinarians will benefit in terms of an effective antimicrobial treatment for dairy cows with mastitis. Human health risks along with major economic losses may be reduced as more knowledge is gained related to the prevalence and implications of MRSA.

Explanation of abbreviations:
1. *mec*A encodes for methicilin resistance[6]
2. *coa* encodes for the enzyme coagulase produced by all *Staph aureus* and is not produced by non-*Staph. aureus* strains[3]
3. Staphylococcus genus specific 16s rRNA[6]
4. *nuc* encodes for the thermostable nuclease of Staph. aureus[6]
5. *mup* encodes for mupirocin resistance and is significant because mupirocin is the last defense against MRSA infections[6]

Introduction

- **Mastitis is an inflammation in the mammary gland. 30% of cases in dairy cows are caused by *Staphylococcus aureus (Staph. aureus).***
- **Mastitis is very costly and the dairy industry loses $80-$125 per cow per year[1] and $2 billion nationwide[5]. These costs include treatment, vet bills, culled animals, and a decrease in milk yield.**

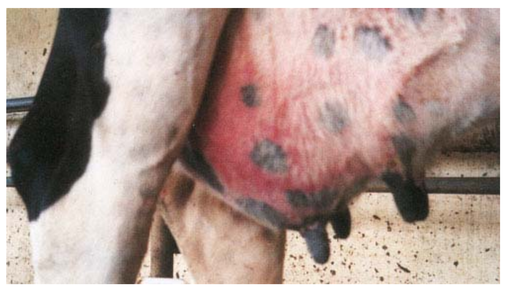


Figure 1. A mastitic dairy cow

- **Over time, *Staph. aureus* has gained resistance to β-lactam antibiotics due to production of a penicillin-binding protein that possesses low affintiy to β-lactams. This protein is encoded by the *mec*A gene in methicillin-resistant *Staph. aureus or MRSA*[4] .**
- **MRSA can cause mastitis in dairy cows, however, it is rarely reported or studied in the US. Literature is mostly available on studies in Europe and Korea.**

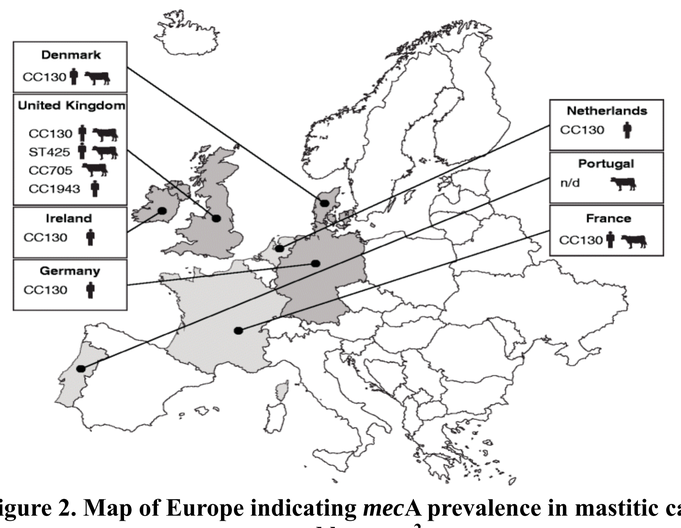


Figure 2. Map of Europe indicating *mec*A prevalence in mastitic cattle and humans[2]

- **Investigating the prevalence of MRSA in mastitic dairy cows in the United States is significant in light of major economic losses to the industry. There is also a public health concern regarding those who consume raw milk and products manufactured with raw milk.**
- **The objective of this study is to investigate the prevalence of MRSA in milk samples from mastitic dairy cows from dairy fars in Ontario and Chino, CA.**
- **It is hypothesized that there will be a low prevalence of MRSA in the milk samples collected.**

Materials and Methods

- **318 milk samples were collected from mastitic dairy cows in San Bernardino Country from 2007-2011 and stored frozen at -20ºC.**
- ***Staph. aureus* strains positive and negative for methicillin resistance as well as various other bacteria strains, serve as controls for this experiment.**
- **<u>Isolation of *Staph aureus:*</u> milk samples were streaked on sterile blood agar (5/10% blood) to isolate bacterial colonies. Representative colonies grown on the blood agar were purified on tryptic soy agar. Using microscopy, purified colonies were observed to determine if the isolates are gram positive cocci, characteristic of *Staph. aureus.* To further identify *Staph. aureus*, the standard catalase test was carried out on each purified colony.**

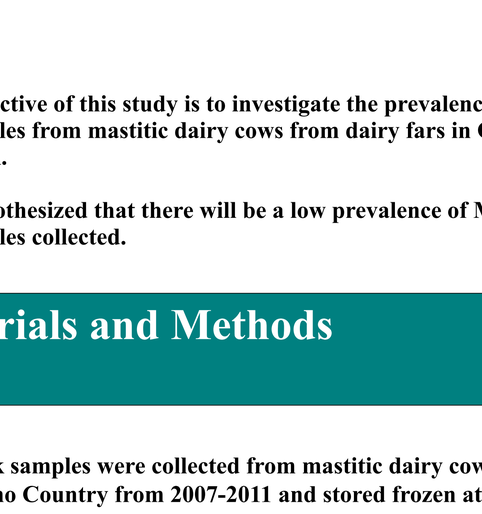


Figure 3. Isolate Growth on Triptic Soy Agar

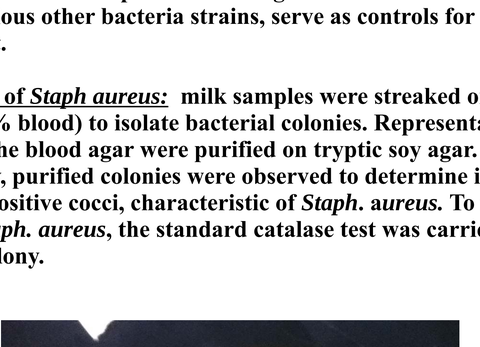


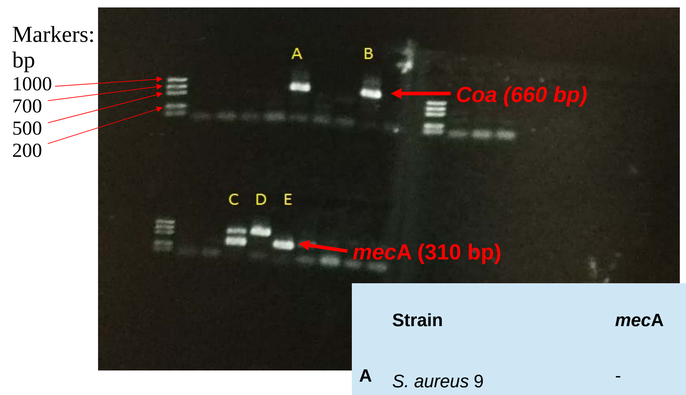
Figure 4. Gram positive cocci

- **<u>Confirmation of *Staph. aureus* and MRSA isolates:</u> In order to confirm isolates as *Staph. aureus* or MRSA, amplification of unique DNA fragments associated with *Staph. aureus* and MRSA will be performed using PCR. A duplex PCR assay was developed to amplify *mec*A and *coa.* A multiplex PCR assay as described by Zhang et al.[6] will be used to amplify *Staphylococcus* genus specific 16s rRNA, *nuc*, and *mup.***
- **Duplex Thermal Cycling Protocol:**
  Initial denaturation will be conducted at 94ºC for 5 mins followed by 35 cycles of 94ºC for 1 min, 58ºC for 1 min, and 72ºC for 2 mins; and final extension at 72ºC for 10 min.
- **Multiplex Thermal Cycling Protocol:**
  Initial denaturation will be conducted 94ºC for 5 min, followed by 10 cycles of 94ºC for 40 sec, 68ºC for 40 sec, and 72ºC for 1 min; 25 cycles of 94ºC for 1 min, 58ºC for 1 min, and 72ºC for 2 min; and final extension at 72ºC for 10 min
- **Detection of PCR products will be determined by agarose gel electrophoresis. 2% (wt./vol) gels will be stained with ethidium bromide and fragment sizes will be determined.**
- **20 quality control strains will serve as positive and negative controls for the genes amplified through PCR. Anticipated molecular weights of each fragment are as follows;**
  - ***mec*A (310 bp) [6]**
  - ***coa* (660 bp)[3]**
  - ***Staphylococcus* genus specific 16s rRNA (756 bp)[6]**
  - ***nuc* (279 bp)[6]**
  - ***mup* (457 bp)[6]**
- **Negative quality control strains include *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Samonella braenderup, Listeria innocua, Listeria welshia, Listeria seelgi, Escherichia coli*, and 7 non-pathogenic *Escherichia coli* strains.**
- **Positive quality control strains include 5 *Staph. aureus* strains.**
- **Isolates confirmed as *Staph. aureus* or MRSA through analysis of PCR products will be further analyzed by conducting antibiotic resistance profiles. These profiles will include antibiotics such as cefoxitin, oxacillin, methicillin, and mupirocin.**

Preliminary Results

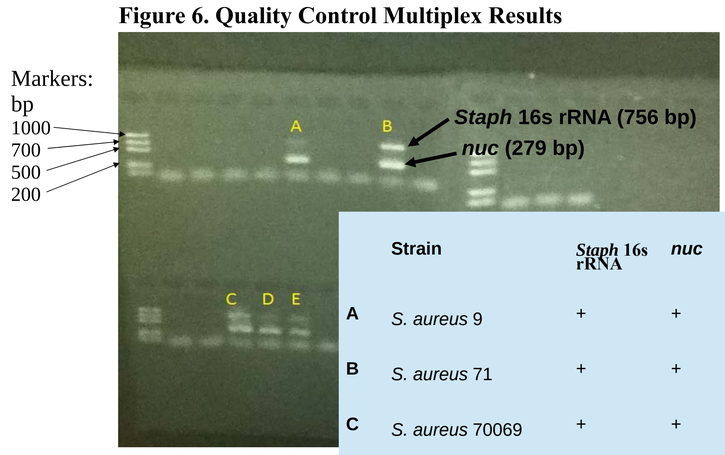
- **Through the preliminary tests conducted, there are 71 presumptive *Staphylococcus* isolates, approximately 22% of the samples.**
- **Anticipated fragment sizes of the positive quality control strains were observed. There are no false positives and one false negative. *Staph. aureus* 43300 was *coa* negative when it was expected to be positive. All negative quality control strains were negative.**

Figure 5. Quality Control Duplex Results



	Strain	*mec*A	*coa*
A	*S. aureus* 9	-	+
B	*S. aureus* 71	-	+
C	*S. aureus* 70069	+	+
D	*S. aureus* 29213	-	+
E	*S. aureus* 43300	+	-

Figure 6. Quality Control Multiplex Results



	Strain	*Staph* 16s rRNA	*nuc*	*mup*
A	*S. aureus* 9	+	+	-
B	*S. aureus* 71	+	+	-
C	*S. aureus* 70069	+	+	-
D	*S. aureus* 29213	+	+	-
E	*S. aureus* 43300	+	+	-

Discussion

- **Both PCR assays will be conducted for the 71 presumptive *Staphylococcus* isolates in order to confirm the prevalence of *Staph. aureus* or MRSA. The quality control data will serve as positive and negative controls for PCR products of each isolate. Negative quality controls eliminate what is negative in the results, further validating the PCR assays. Both positive and negative controls will aid with interpretation of data from unknown samples.**
- **Confirmation of any of these 71 *Staphylococcus* isolates as MRSA will indicate an immense need for further research on this family of pathogens, in California and in the US.**

Literature Cited

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