Housing, bedding, and mastitis risk: Update on bedding and mastitis studies in Vermont.

- John Barlow DVM PhD
- Caitlin Jeffrey DVM
- Ashma Chakrawarti MS
- Heather Schuettner
- Theresa Astmann

- Department of Animal and Veterinary Sciences
- University of Vermont

The UNIVERSITY of VERMONT

- What's new in mastitis and mastitis control?
  - Highlights from the 2023 National Mastitis Council Meeting
  - Some emerging bacterial species you may starting hearing about

- Does Staphylococcus aureus develop resistance to cinnamon bark oil?
- What is the winter housing and bedding situation on organic dairy farms in Vermont?
- Does the choice of housing and bedding impact mastitis risk?
- Antibiotic resistant *Staphylococcus aureus* in people on dairy farms?

Highlights from 2023 Nation Mastitis Council Meeting

- Public concerns about dairy cattle welfare
- Earning public trust and the social license to operate
- Labor work force and navigating social responsibilities

Highlights from 2023 Nation Mastitis Council Meeting

- Public concerns about dairy cattle welfare

Confinement

↓

Tie-stalls

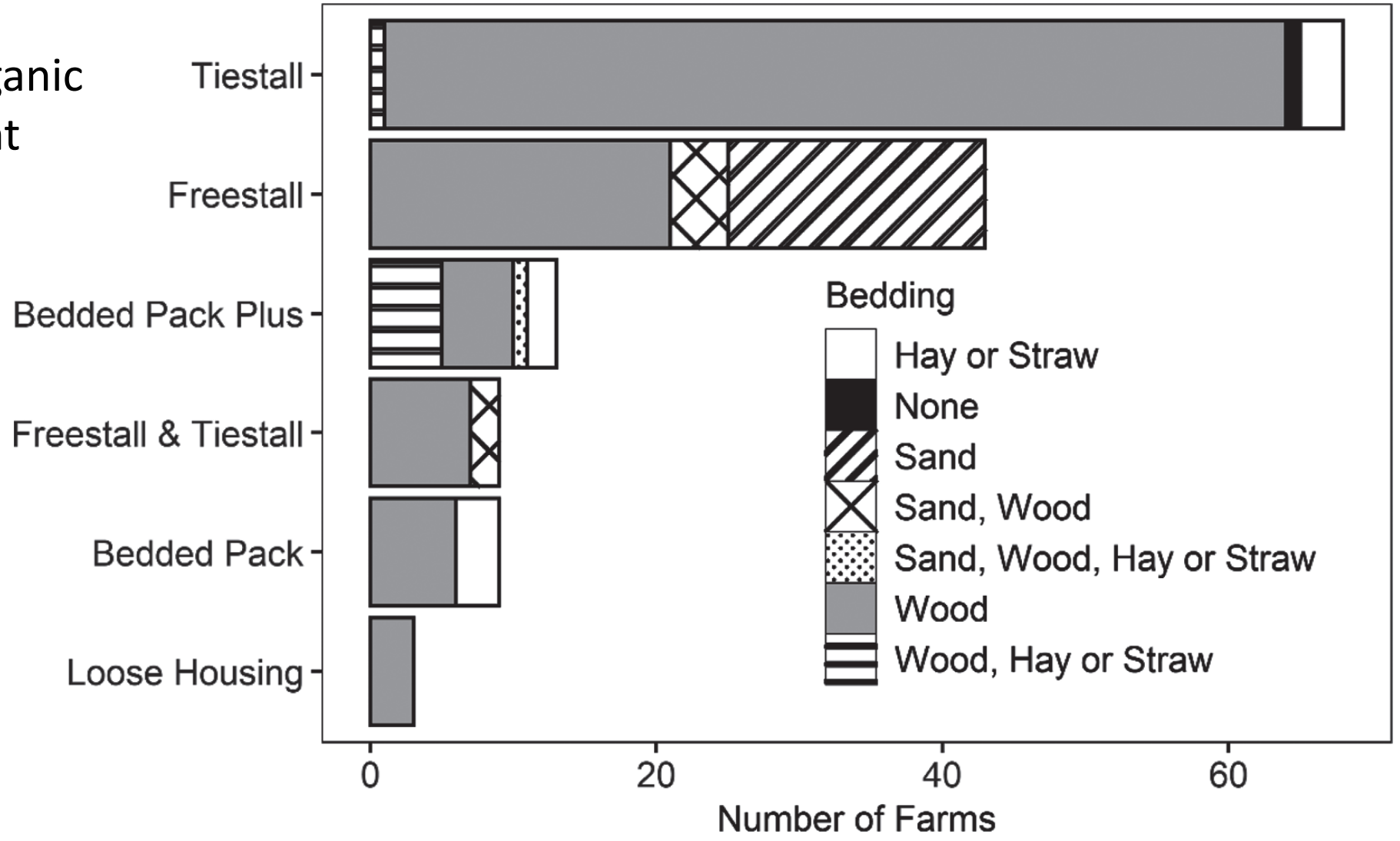
Pasture access

Early separation of cows and their calves

2019 survey quantifying winter housing and bedding types used on Vermont organic dairy farms

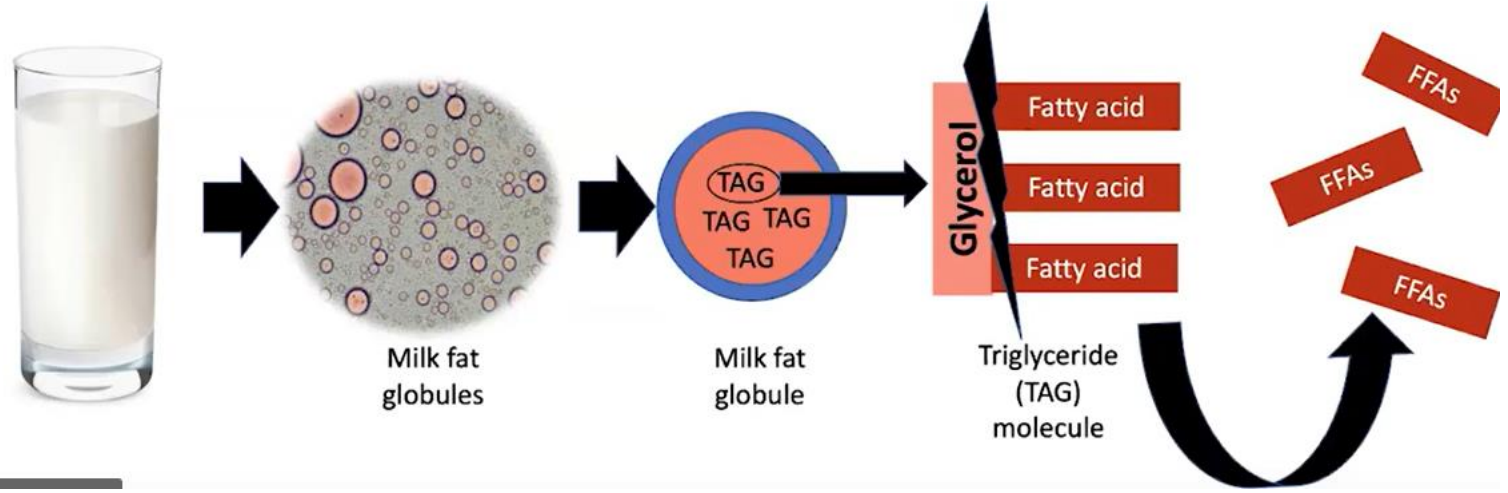
177 certified organic farms in Vermont

148 (84%) responded



Free Fatty acids - Woodhouse and Kelton, Guelph

Mechanical lipolysis
Bacterial lipolysis



- off-flavor
- rancidity
- reduced foaming ability,
- inhibited milk fermentation

Factors associated with increased FFAs

- No Additional in line cooling
- Less frequent filter changes

Highlights from 2023 Nation Mastitis Council Meeting

Characterization of Antibacterial Activity of *Staphylococcus chromogenes* Isolates Originating from Dairy Cattle

Isensee and Adkins, Missouri

S. chromogenes – beneficial commensal or pathogen?

378 *S. chromogenes* isolates that originated from 25 different dairy farms from 6 states

3% demonstrated ability to inhibit *Staphylococcus aureus*

Some “emerging” mastitis pathogens

Lactococcus species

Mammaliicoccus species

Staphylococcus chromogenes

NASM – previously CNS

MALDI-TOF

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

J. Dairy Sci. 103:1785–1794
https://doi.org/10.3168/jds.2018-16199

Distribution of *Lactococcus* spp. in New York State dairy farms and the association of somatic cell count resolution and bacteriological cure in clinical mastitis samples

J. C. Scillieri Smith,[1] P. Moroni,[2,3] C. G. Santisteban,[2] B. J. Rauch,[2] P. A. Ospina,[2*] and D. V. Nydam[2]
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[3]Dipartimento di Medicina Veterinaria, Università degli Stuc

biology

MDPI

Article

Mammaliicoccus spp. from German Dairy Farms Exhibit a Wide Range of Antimicrobial Resistance Genes and Non-Wildtype Phenotypes to Several Antibiotic Classes

Tobias Lienen *, Arne Schnitt, Jens Andre Hammerl, Sven Maurischat and Bernd-Alois Tenhagen *

Rosa et al. Veterinary Research (2022) 53:84
https://doi.org/10.1186/s13567-022-01102-4

RESEARCH ARTICLE

Species identification by MALDI-TOF MS and *gap* PCR–RFLP of non-*aureus* *Staphylococcus*, *Mammaliicoccus*, and *Streptococcus* spp. associated with sheep and goat mastitis

Nives Maria Rosa[1†], Martina Penati[2,3†], Sara Fusar-Poli[2,3], Maria Filippa Addis[2,3*] and Sebastiana Tola[1]

Background – endogenous inhibitors

Isolates from the microbiota of the healthy cow skin (commensal bacteria) have antimicrobial activity against bovine mastitis pathogens

Simultaneous antagonism or deferred antagonism assays

Da Costa, L. (2017). Why mastitis treatments fail. Retrieved from https://www.farmanddairy.com/columns/why-mastitis-treatments-fail/417652.html

28 of 284 isolates inhibitory to mastitis pathogens “Corynebacterium” *Arcanobacterium haemolyticum* and *Trueperella pyogenes*, *Bacillus*, *Aerococcus*, *Staphylococcus* (Woodward et al., 1987)

33 inhibitors (9 strong) from 2800 isolates from teat swabs and milk cultures; 20 Gram-positive cocci and 13 Gram-positive bacilli; Focused on the bacilli, species id by 16S rRNA gene sequences (789 bp product)
B. pumilus, B. subtilis, B. licheniformis, B. thuringiensis or B. cereus. (Al_Qumber and Tagg, 2006)

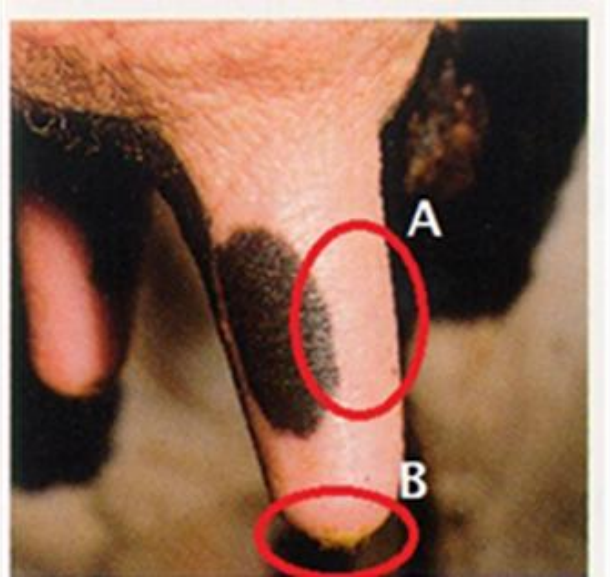
2 of 10 *S. chromogenes* from teat apex swabs (De Vliegher et al., 2004)

38 (7 strong) out of 254 isolates from teat apex swabs; 12 different non-*aureus Staphylococcus* species(Braem et al. 2014)

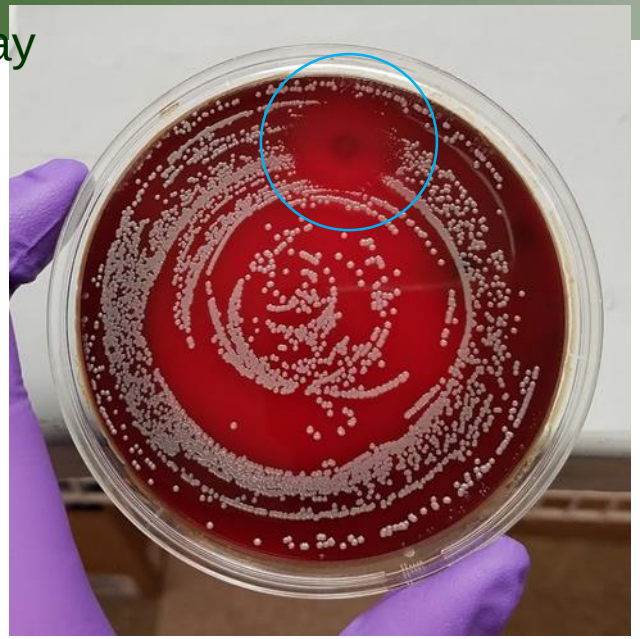
Study Design

- Collected skin swab samples from 8 lactating Holstein cows at the UVM Teaching herd – 50 cow herd, housed in tie-stalls, milked in a parlor, all milkers wear nitrile gloves, chlorhexidine pre- and post-milking teat disinfectant
  - 4 cows with composite milk SCC < 60,000 cells/mL for 5 consecutive months
  - 4 cows with composite milk SCC >280,000 cells/mL for 4 to 5 consecutive months

- Swabbed Three locations: teat barrel (TB), teat end (TE), streak canal (SC)
- 96 total samples
- Samples stored -20°C for up to 4 weeks

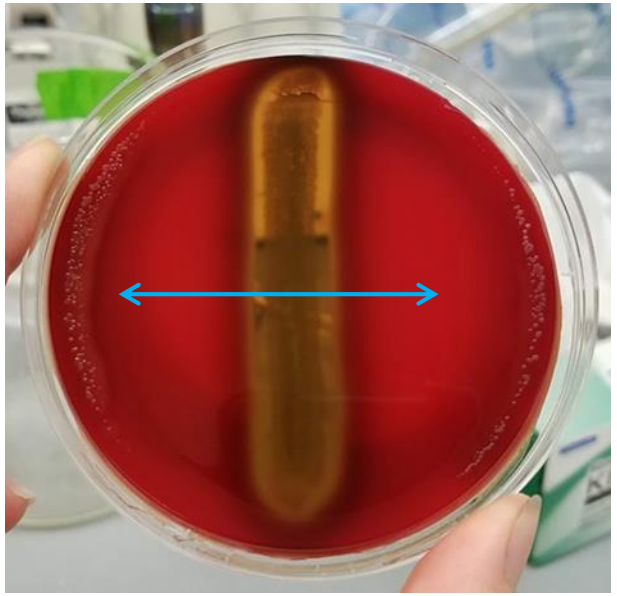


Screening assay

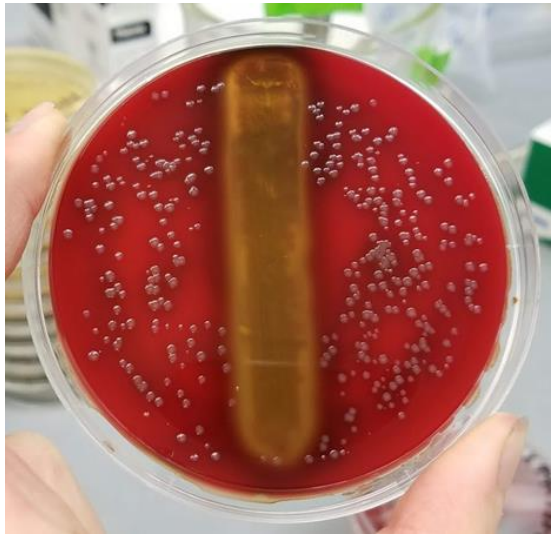


Quantify the number of isolates observed per total cfu/ml plated

Simultaneous antagonism assay



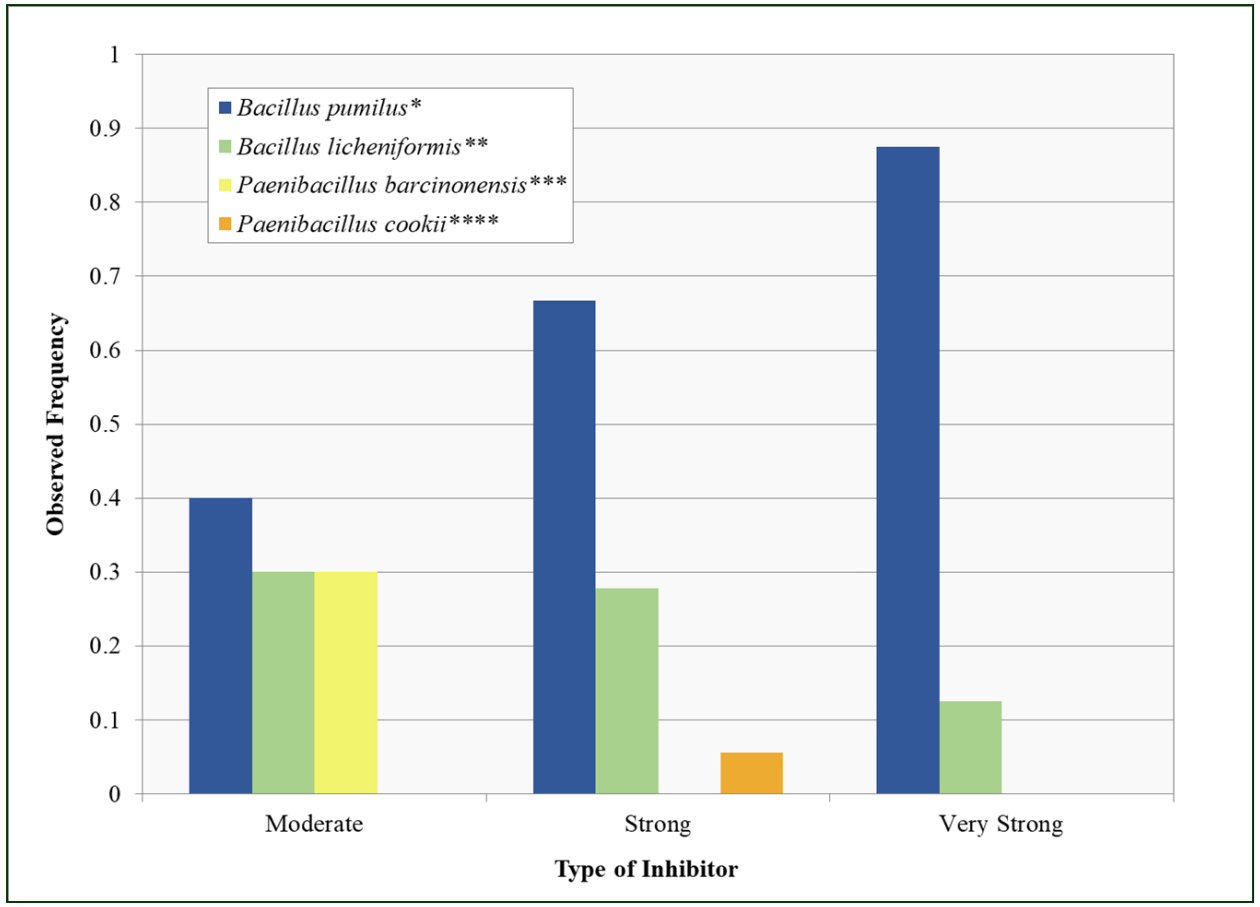
Quantify the size of the zone of inhibition for each isolate



Test plate

Controls

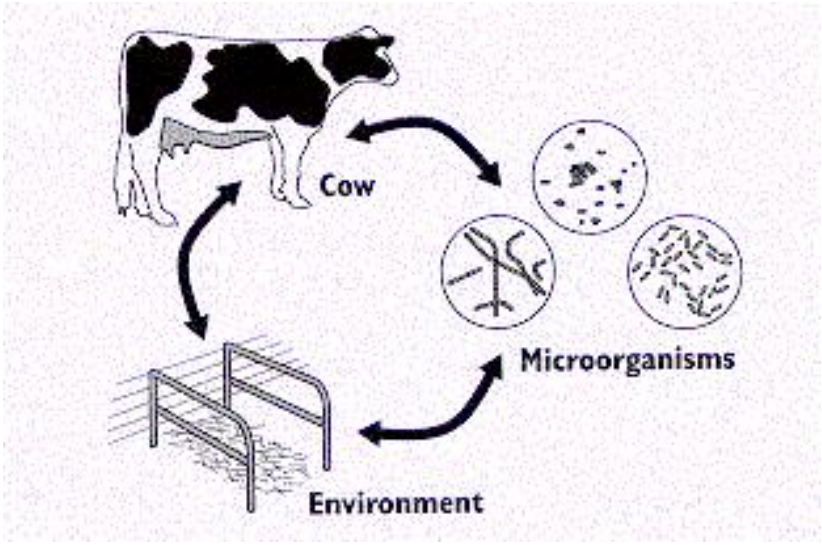
Species Identified by extent of inhibition



Moderate: width of zone of inhibition 3 cm or less (n = 10)
Strong: width of zone of inhibition between 3 and 5 cm (n = 18)
Very strong: width of zone of inhibition greater than 5 cm, or extremely minimal/completely absent SA CP20 growth (n = 8)

- Bedding and Mastitis

the environment as a source of organisms causing infections



<u>Environmental Mastitis</u>

Coliform organisms

E. coli and *Klebsiella*

Environmental Streptococci

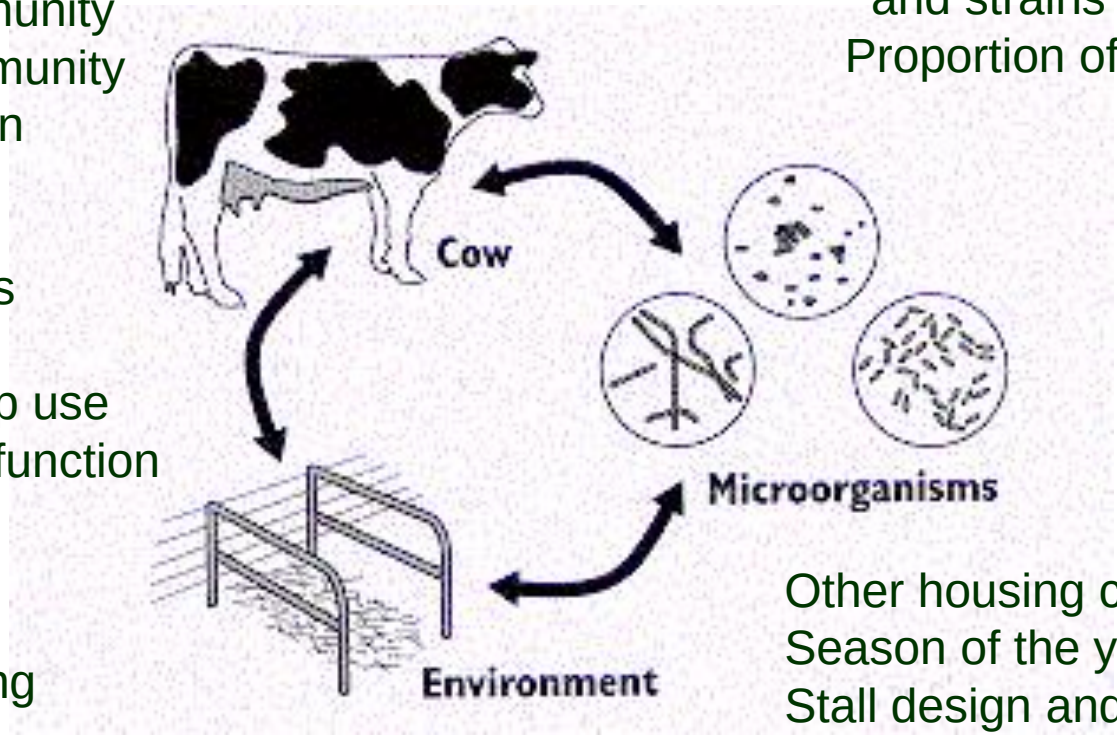
Streptococcus uberis

Stage of lactation
Lactation number
Nutrition and Immunity
Genetics and Immunity
Teat end condition

Udder cleanliness
Milking Hygiene
pre and post dip use
Milking machine function

Type of bedding

Frequency of bedding changes



Dominant species
and strains of bacteria
Proportion of herd infected

Other housing conditions
Season of the year
Stall design and stall use
Stocking densities
Feeding practices

The level of pathogen exposure influences mastitis risk

Bedding and Mastitis

Clinical or subclinical mastitis

↑

Bacteria in milk or mammary gland

↑

Bacteria on teat skin

↑

Bacteria in bedding

Truth: The impact of bedding, and cleanliness of the udder on rates of mastitis is unclear

Common sense: ***clean, dry, and comfortable***

Mastitis associated organisms

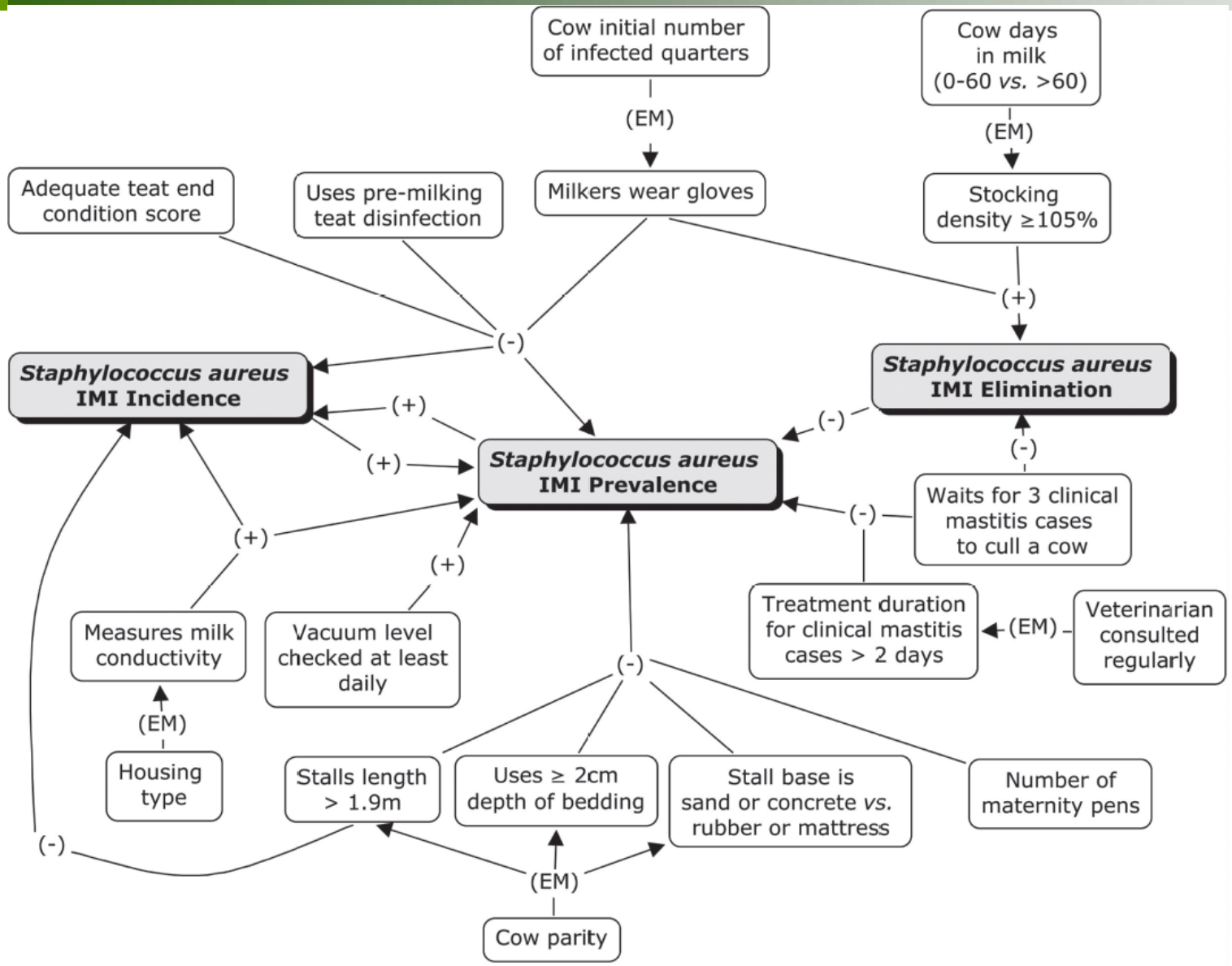
- *Streptococcus* and *Streptococcus*-like organisms (SSLO)
- Gram-negative (coliform) organisms
- *Staphylococcus* species (non-aureus staphylococci)

contagious

- Staphylococcus aureus
- Streptococcus agalactiae

Bedding culture measures

- *Streptococcus* and *Streptococcus*-like organisms (SSLO)
- Coliforms
- Klebsiella spp.
- Non-coliform gram-negative organisms
- *Staphylococcus* species (non-aureus staphylococci)
- Bacillus spp.
- Prototheca spp.



(-):Associated with lower *Staph aureus* IMI incidence, elimination, or prevalence
(+):Associated with higher Staph aureus IMI incidence, elimination, or prevalence
(EM):Effect modifier

http://dx.doi.org/ 10.3168/jds.2011-4711

J. Dairy Sci. 95:1283–1300
http://dx.doi.org/10.3168/jds.2011-4711

Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows

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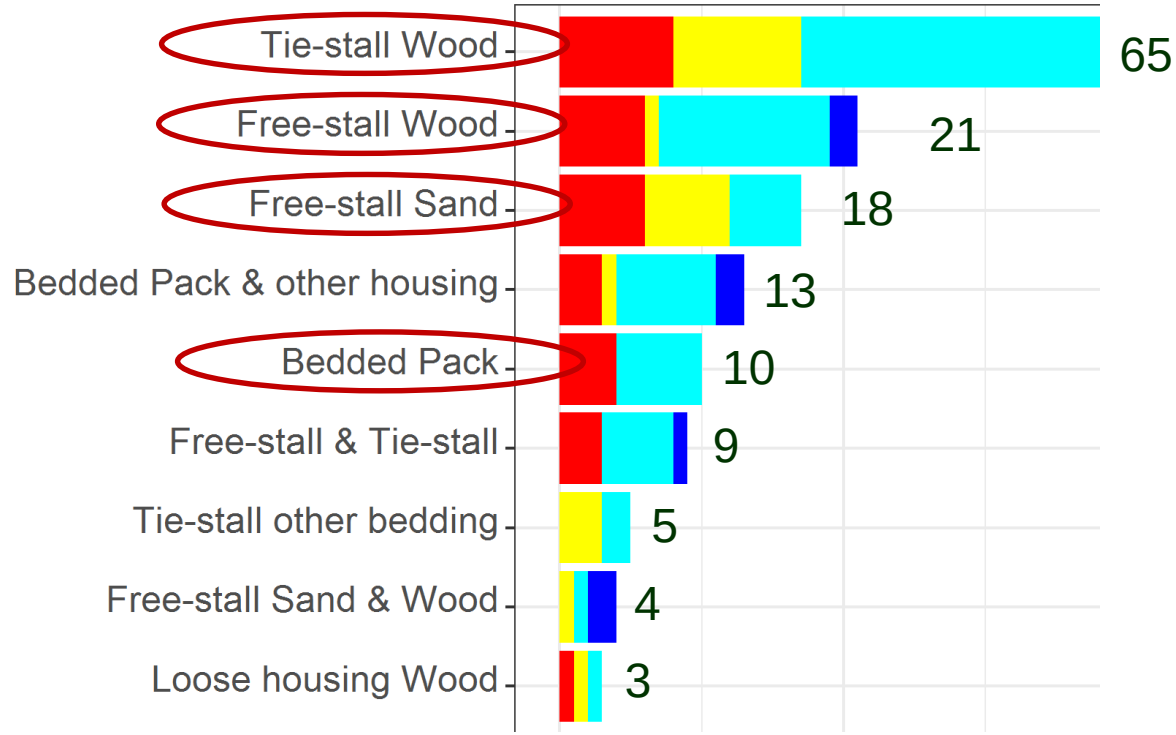
Observational study 2 – cross sectional survey

stratified random design for herd selection in 4 housing categorie

40 herds -10 herds per category
winter herd visit

sample collection
- bedding
- bulk tank milk
- clinical samples
- farm measures

questionnaire



Observational study 3 – case:control longitudinal study

5 case herds – bedded pack -
5 control herds – tie-stall
winter herd visits – 5 months

sample collection
- bedding
- bulk tank milk
- quarter milk sample
- farm measures

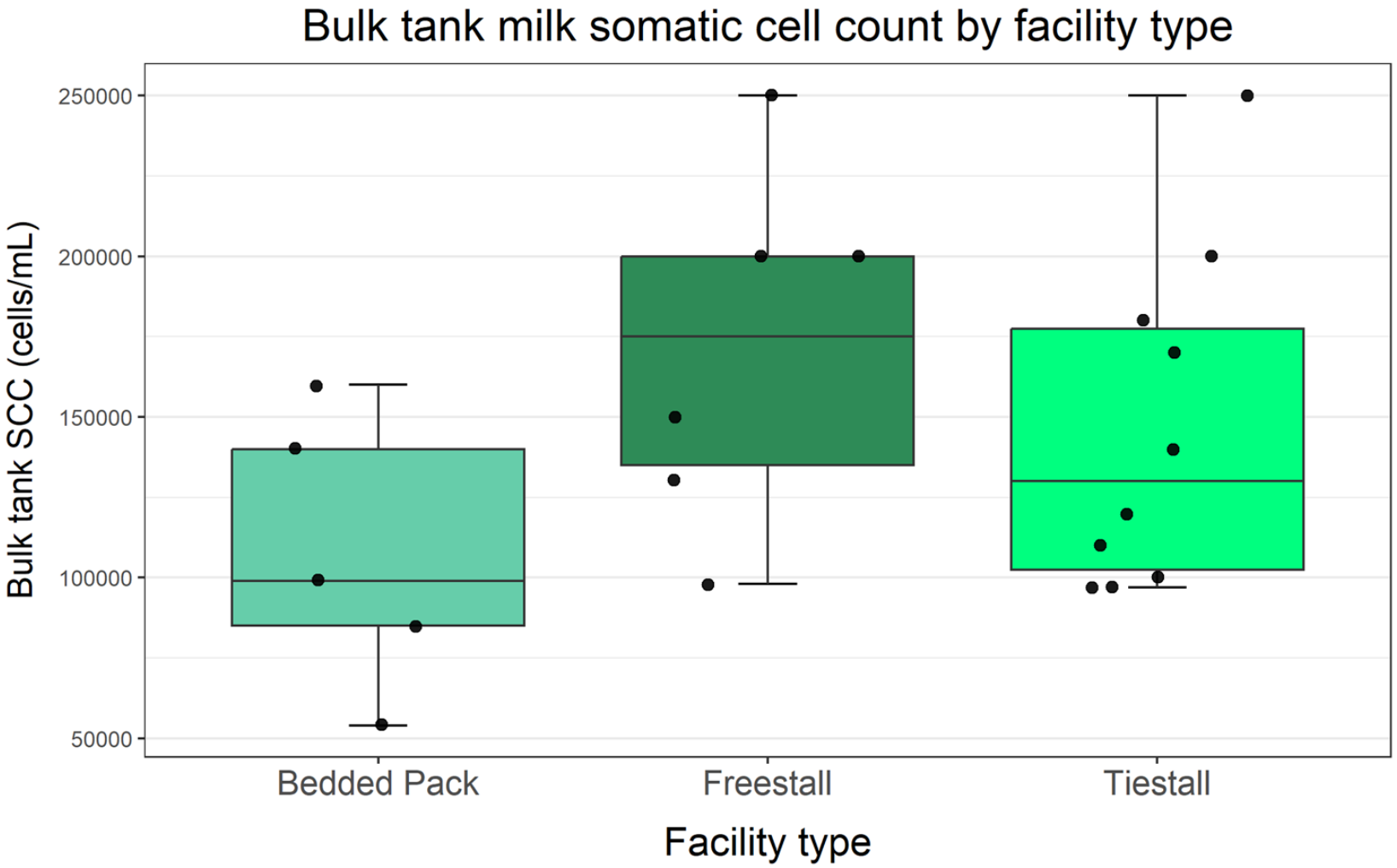
questionnaire

Observational study 2 – cross sectional survey

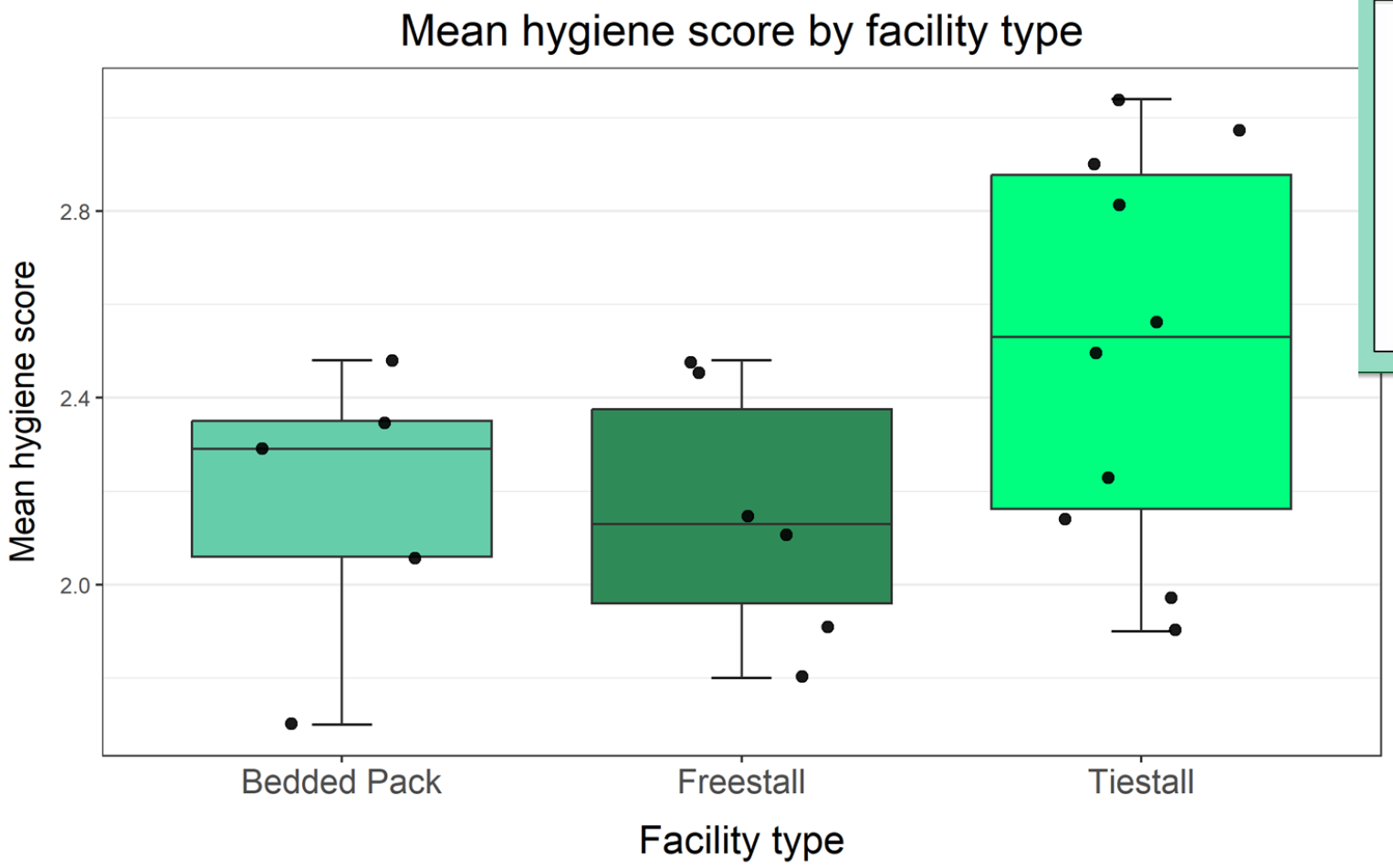
Enrolled 21 herds (of 40 target) pre-covid

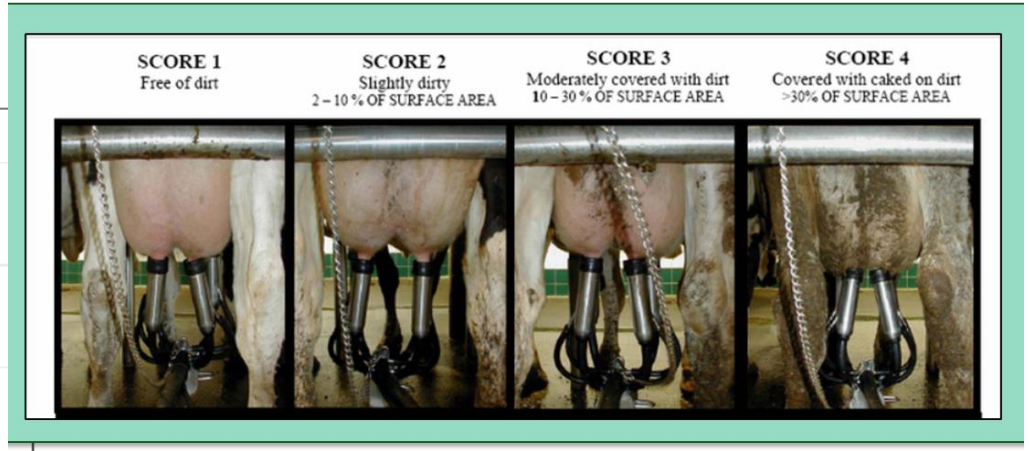
Tie stall wood – 10
Free stall wood - 5
Free stall sand -1
Bedded-pack - 5

Observational study 2 – cross sectional survey



Observational study 2 – cross sectional survey





Herds with higher hygiene scores have more cows with chronic intramammary infections

Observational study 2 – cross sectional survey

On the tie-stall and free-stall herds

Increasing Depth of bedding is associated with

- better udder hygiene
- lower percent of cows with high SCC

https://causecomfortmatters.com/2016/10/23/for-the-love-of-cows-a-dream-barn-was-built/

Observational study 3 – case:control longitudinal study

5 case herds – bedded pack -
5 control herds – tie-stall
winter herd visits – 5 months

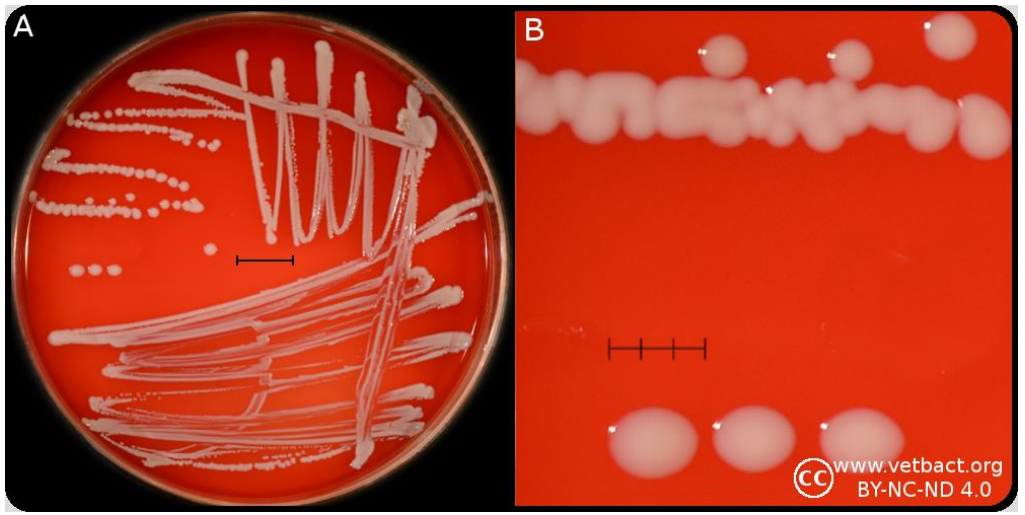
sample collection
- bedding
- bulk tank milk
- quarter milk sample
- farm measures

questionnaire

Results: Overview

- 4,212 quartermilk samples
  - 384 cows enrolled for at least one visit

- 1,325 bacterial isolates found
  - 1,276 identified by MALDI-ToF
  - 726 identified as NASM



Results: NASM IMI by species

Species	No. IMI	Species	No. IMI
S. chromogenes	190	*S. hyicus*	3
S. haemolyticus	28	*M. fleuretti*	3
S. simulans	19	*M. sciuri*	2
S. equorum	15	*S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. gallinarum, S. hominis, S. pseudintermedius, S. saprophyticus, S. succinus*	1
S. agnetis	12		
S. devriesei	7		
S. warneri	7		
S. xylosus	5	***Total number NASM IMI:***	300

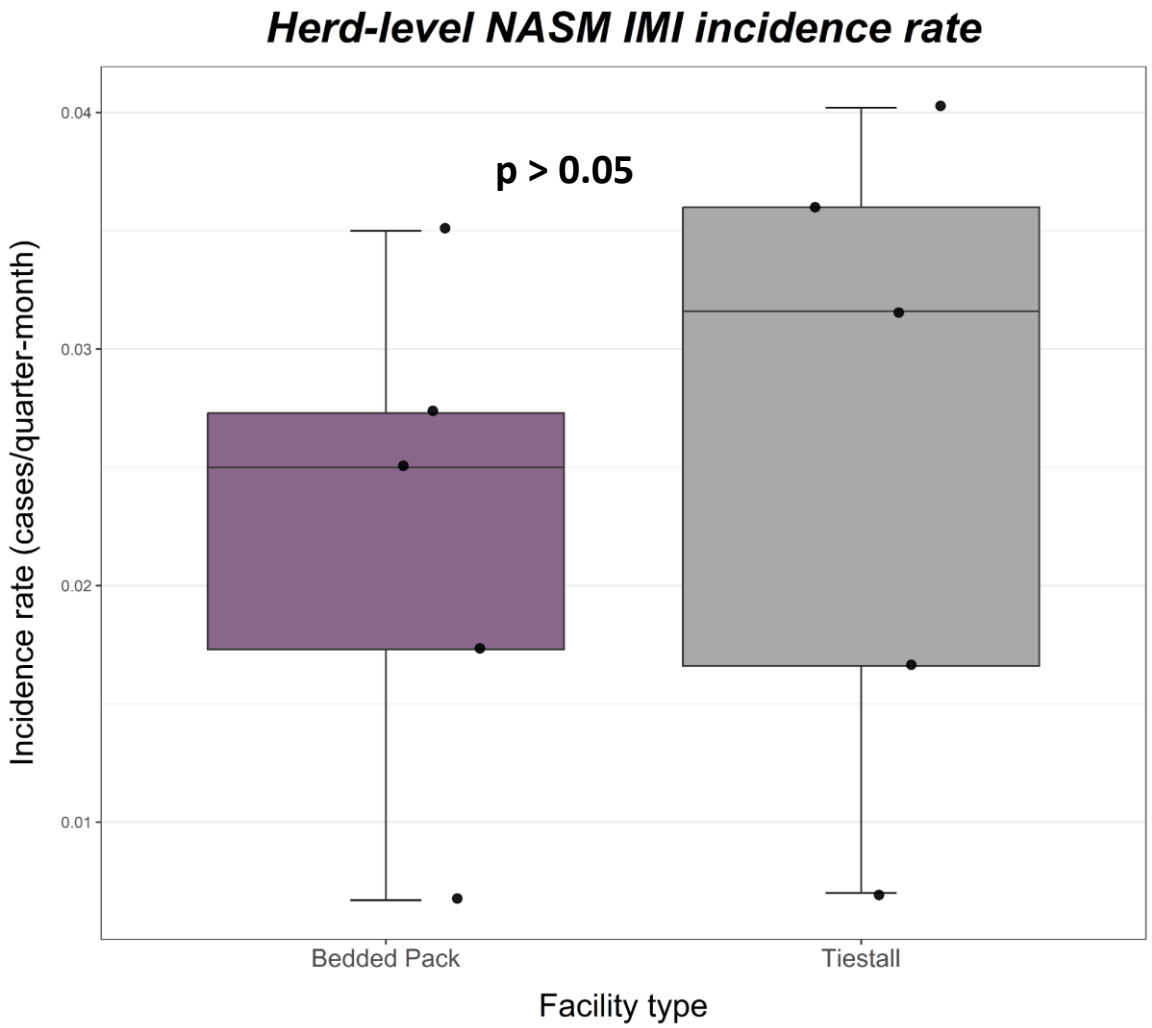


Table 2. Proportion of species causing incident NASM IMI on 10 Vermont organic dairy herds

NASM species	Number of incident IMI	Proportion of incident IMI
Staphylococcus chromogenes	14	38.90%
Staphylococcus equorum	7	19.40%
Staphylococcus haemolyticus	7	19.40%
Staphylococcus simulans	2	5.60%
Staphylococcus agnetis	1	2.80%
Staphylococcus auricularis	1	2.80%
Staphylococcus capitis	1	2.80%
Staphylococcus devriesei	1	2.80%
Mammaliicoccus fleurettii	1	2.80%
Staphylococcus hominis	1	2.80%
Total	**36**	**100.00%**

~ 10 new NASM IMI per 100 cow-months / 10 new cases of subclinical IMI due to NASM each month on a 100-cow dairy

Incidence

Future directions

Are incidence, prevalence, and elimination rates associated with various herd, cow, and quarter-level predictors for both:

- NASM generally, as a group?

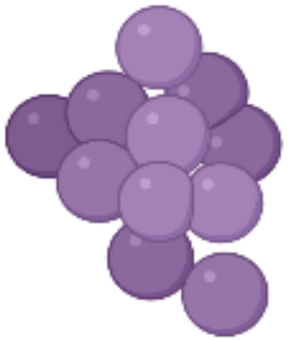
- most frequently-found species or groups of species?

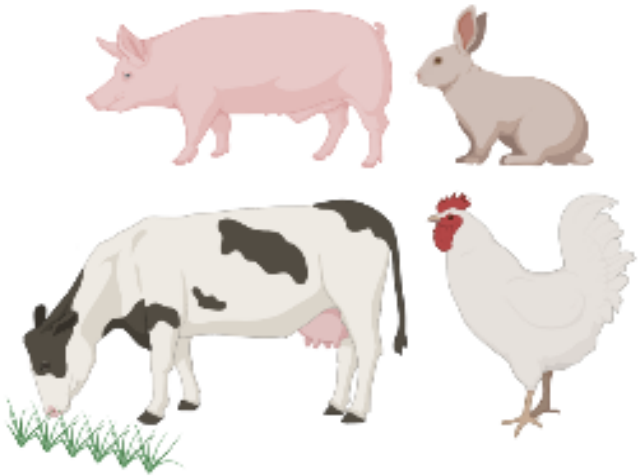
- Herd-level predictors
  - Bedding management practices
  - Milking hygiene practices
  - Bedding and facility type
  - Average hygiene score, prop. dirty udders
- Cow-level predictors
  - Individual hygiene score
  - Parity
  - DIM
- Quarter-level predictors
  - Udder position

Pathogenic strains

Staphylococcus aureus

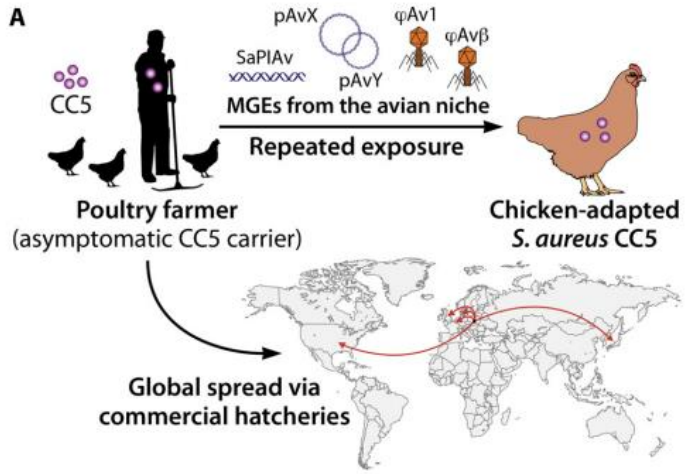
Human associated
CC 8
CC 5
CC 7
CC 30
CC 398

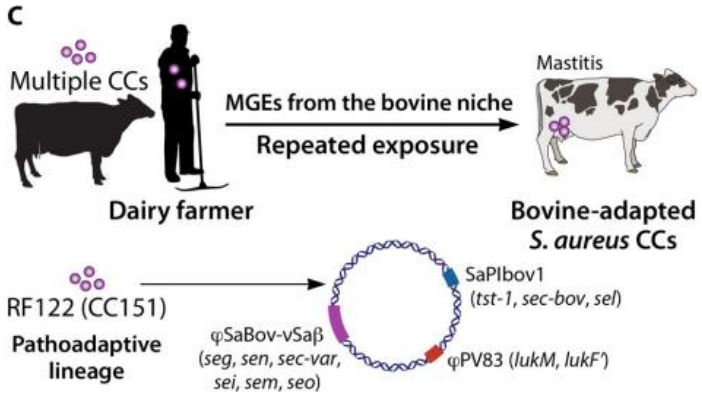




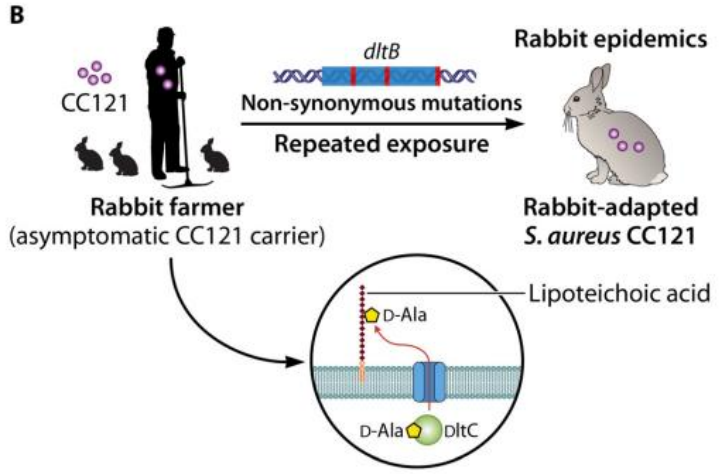
Livestock associated
CC 97
CC 151
CC 352
CC 121
CC 133

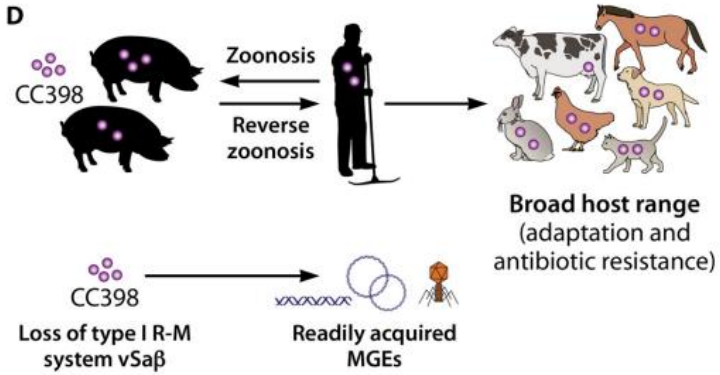
Host jump (spillover) events





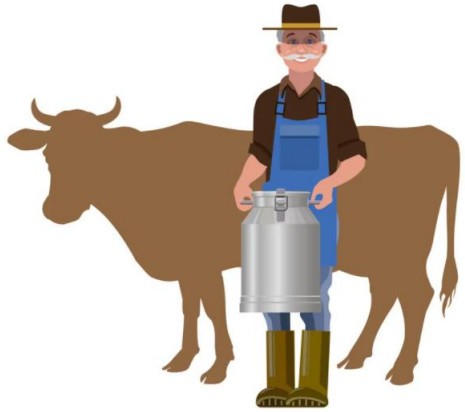
- Loss and gain of host specific virulence genes and antimicrobial resistance genes by mutation or acquisition from mobile genetic elements (MGEs)





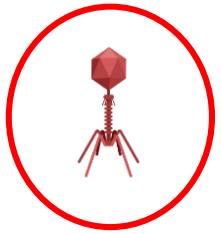
Park S, Ronholm J. 2021. Staphylococcus aureus in agriculture: lessons in evolution from a multispecies pathogen. Clin Microbiol Rev 34:e00182-20. https://doi.org/10.1128/CMR .00182-20

Host jump by *S. aureus* CC8



Human originated
CC8

Loss of prophage (ΦSa3) expressing IEC genes *scn, sak, chp, sea*



Insertion of prophage(ΦSa3) expressing IEC genes *scn, sak, chp, sea* in *hlb gene* locus

Bovine adapted
CC8
RS-PCR GTB*

WGS to compare North American isolates to those of RS-PCR GTB isolates from Europe (*Hans Graber lab)

Staphylococcus aureus **isolates from humans and cattle on Vermont dairy farms making farmstead cheese belong to different clonal complexes**

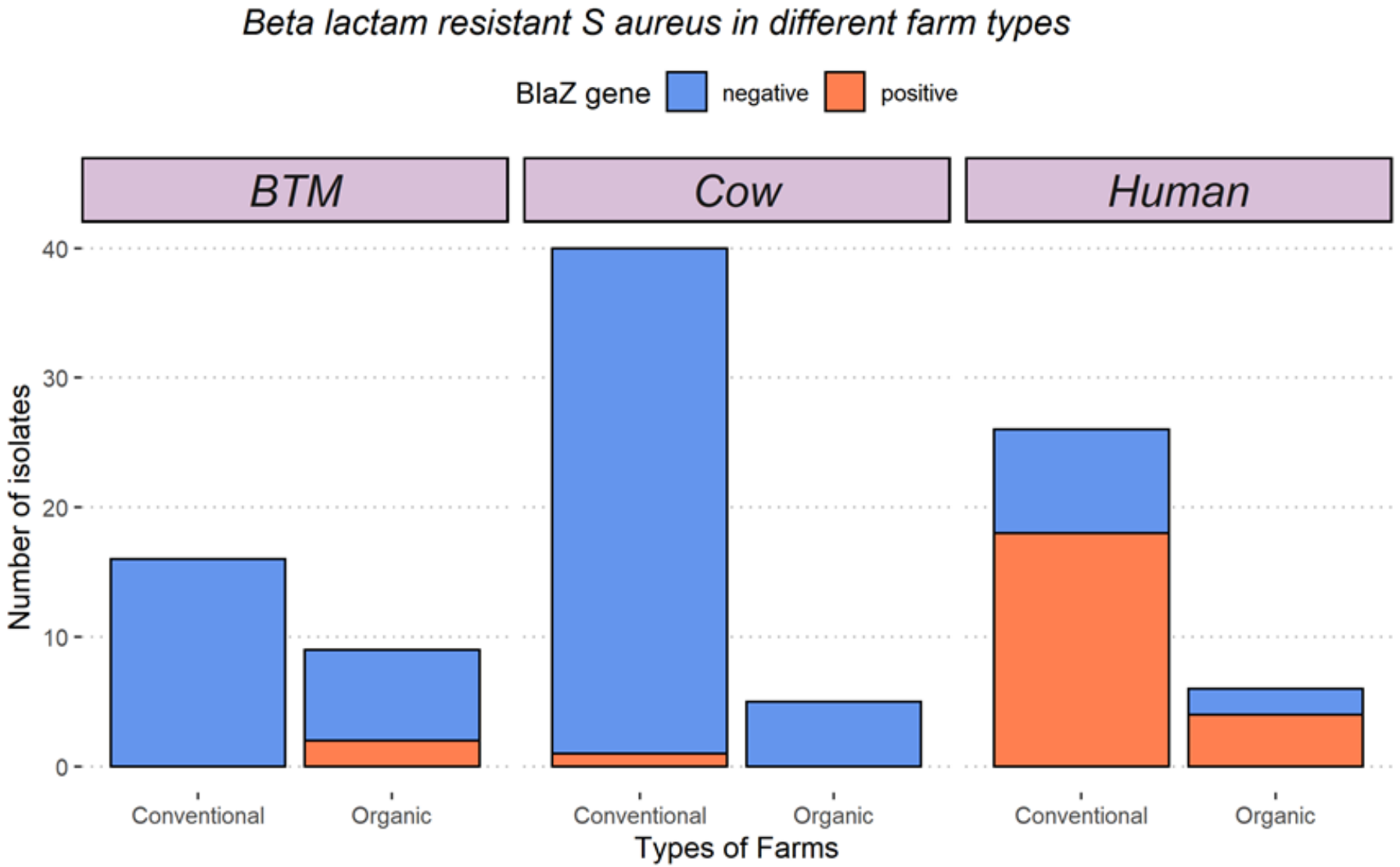
19 farms enrolled

- 4 certified organic
- 15 conventional

Samples collected from

- individual cow quarter milk
- farm bulk tank milk
- human nasal and hand swabs

https://www.jasperhillfarm.com

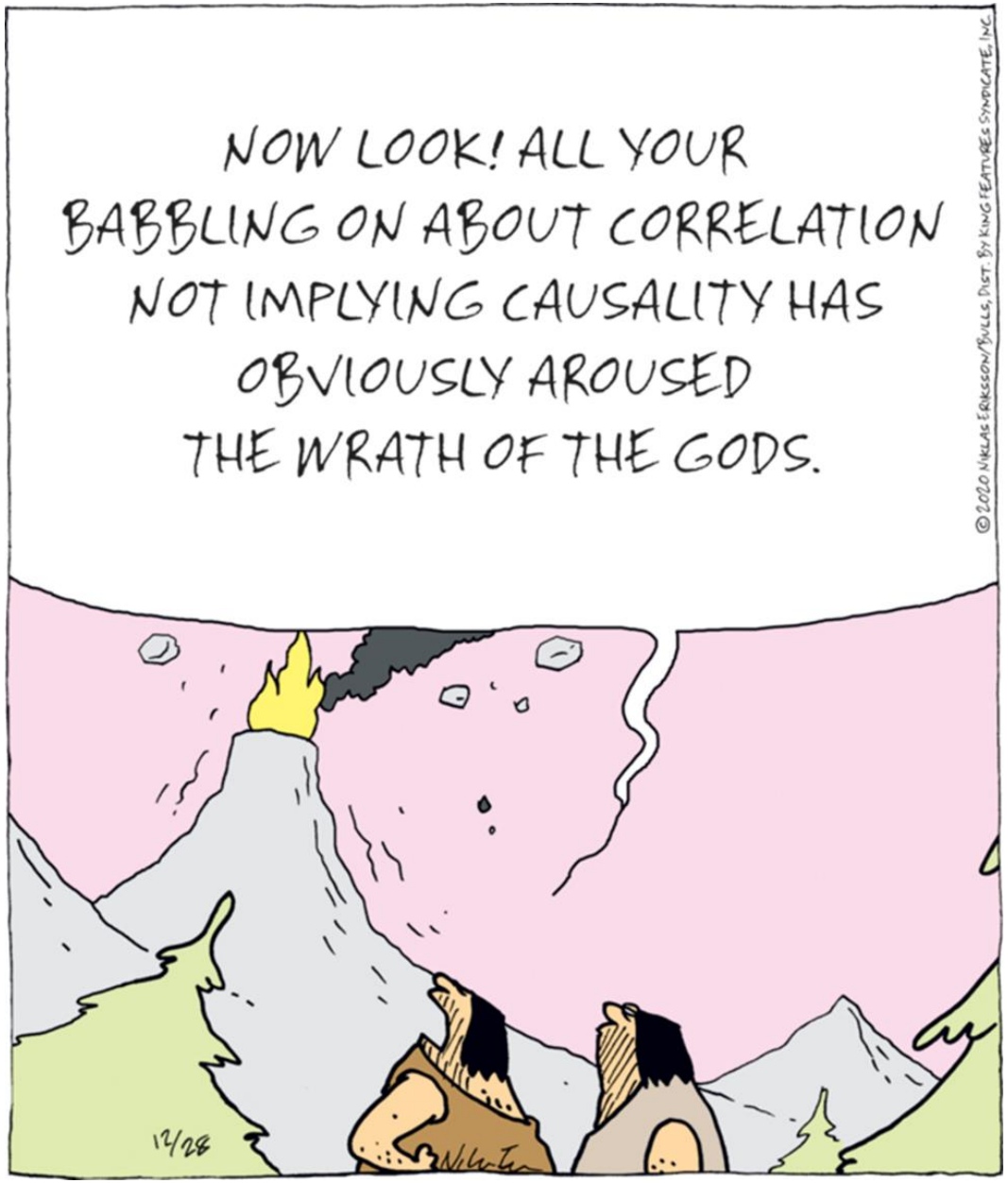

Beta lactam resistant S aureus in different farm types
BlaZ gene
negative
positive
BTM
Cow
Human
Number of isolates
40
30
20
10
0
Conventional
Organic
Conventional
Organic
Conventional
Organic
Types of Farms

- Collaborating Farmers

- Robert Mugabi, DVM PhD
- Christine Casey, DVM MPH
- Ashma Chakrawarti
- Caitlin Jeffrey DVM
- Korin Eckstrom MS
- Pheobe Laguiby
- Ariela Burk
- Amanda Ochoa
- Amanda Carmellini
- Heather Schuettner
- Theresa Astmann

Funding

- USDA HATCH Project VT-H02413MS - UVM Experiment Station Multistate Project NE1748 Mastitis Resistance to Enhance Dairy Food Safety
- USDA Organic Agriculture Research and Extension Initiative grant
- USDA-NIFA Predoctoral Fellowship grant


NOW LOOK! ALL YOUR BABBLING ON ABOUT CORRELATION NOT IMPLYING CAUSALITY HAS OBVIOUSLY AROUSED THE WRATH OF THE GODS.
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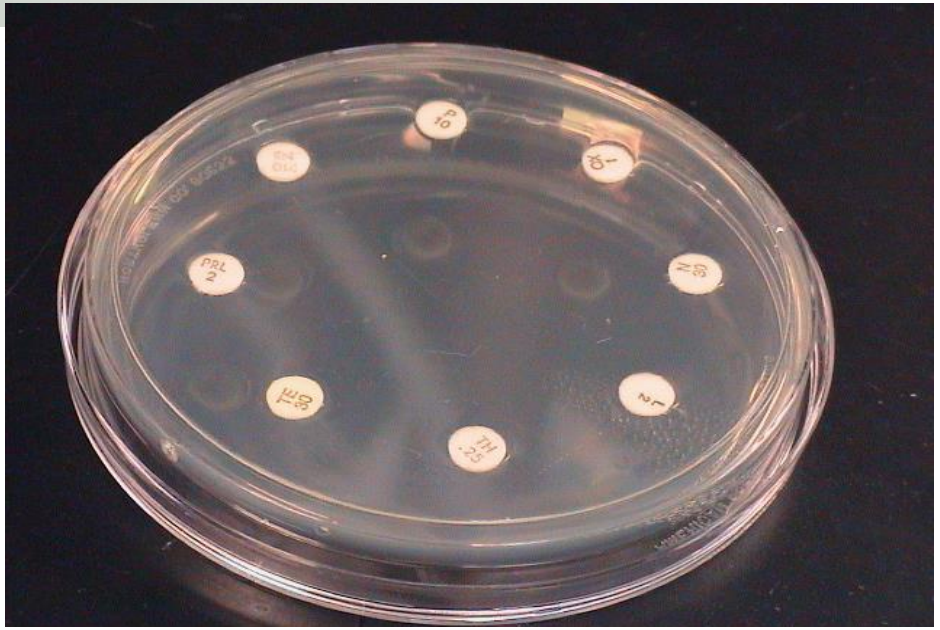
Antimicrobial susceptibility testing

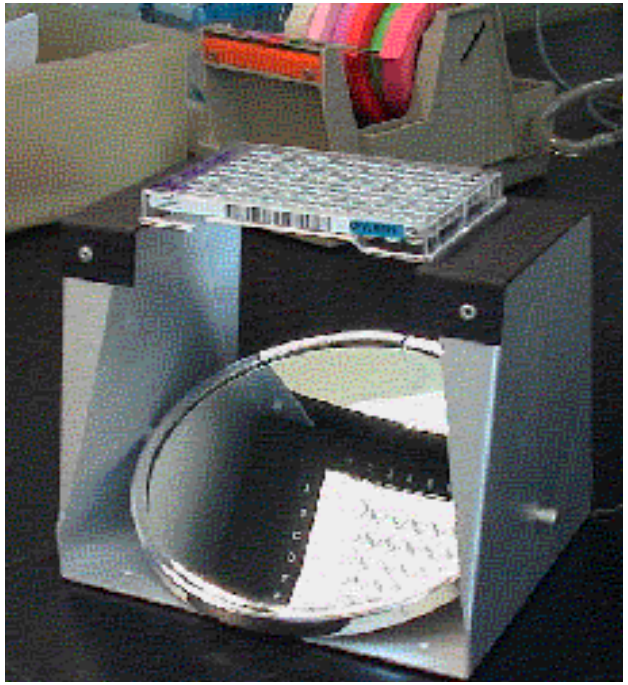
Agar Disc diffusion

ampicillin, penicillin, erythromycin, cephalothin, ceftiofur, pirlimycin, tetracycline, oxacillin, amoxicillin/clavulanic acid, cefoxitin, clindamycin, enrofloxacin, gentamycin, lincomycin, vancomycin, cefazolin, tilmicosin and sulfamethoxazole/trimethoprim



broth microdilution (Sensititre Mastitis MIC plate)

ampicillin, cephalothin, ceftiofur, erythromycin, oxacillin (w/2% NaCl), penicillin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, and tetracycline

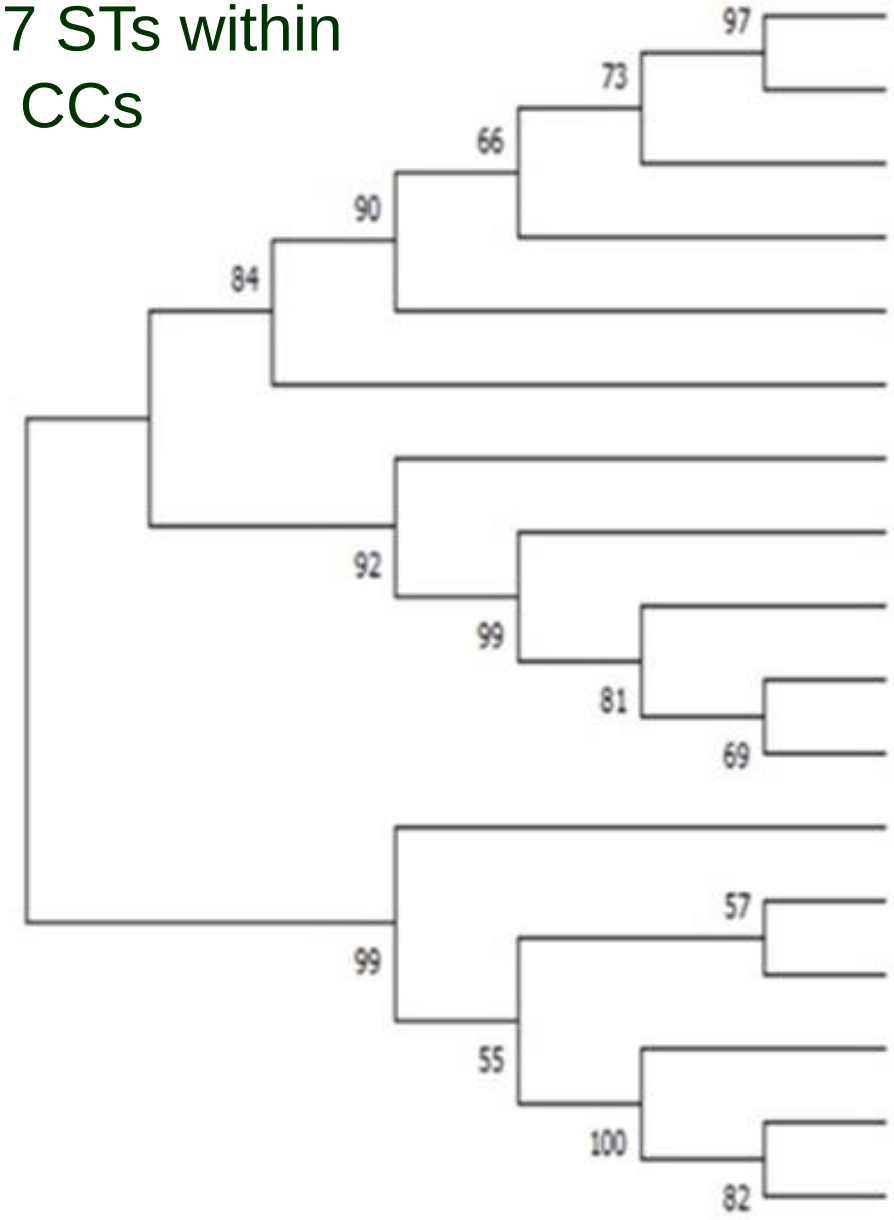


Gene fragment target PCR for

blaZ – beta-lactamase

mecA – methicillin resistance

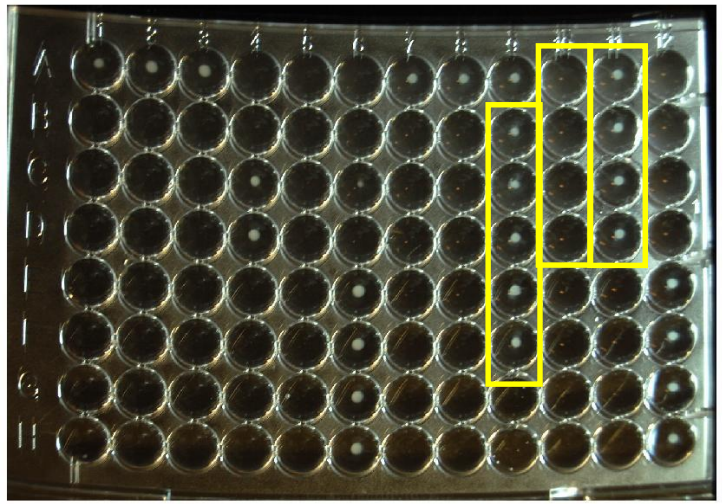
17 STs within 8 CCs

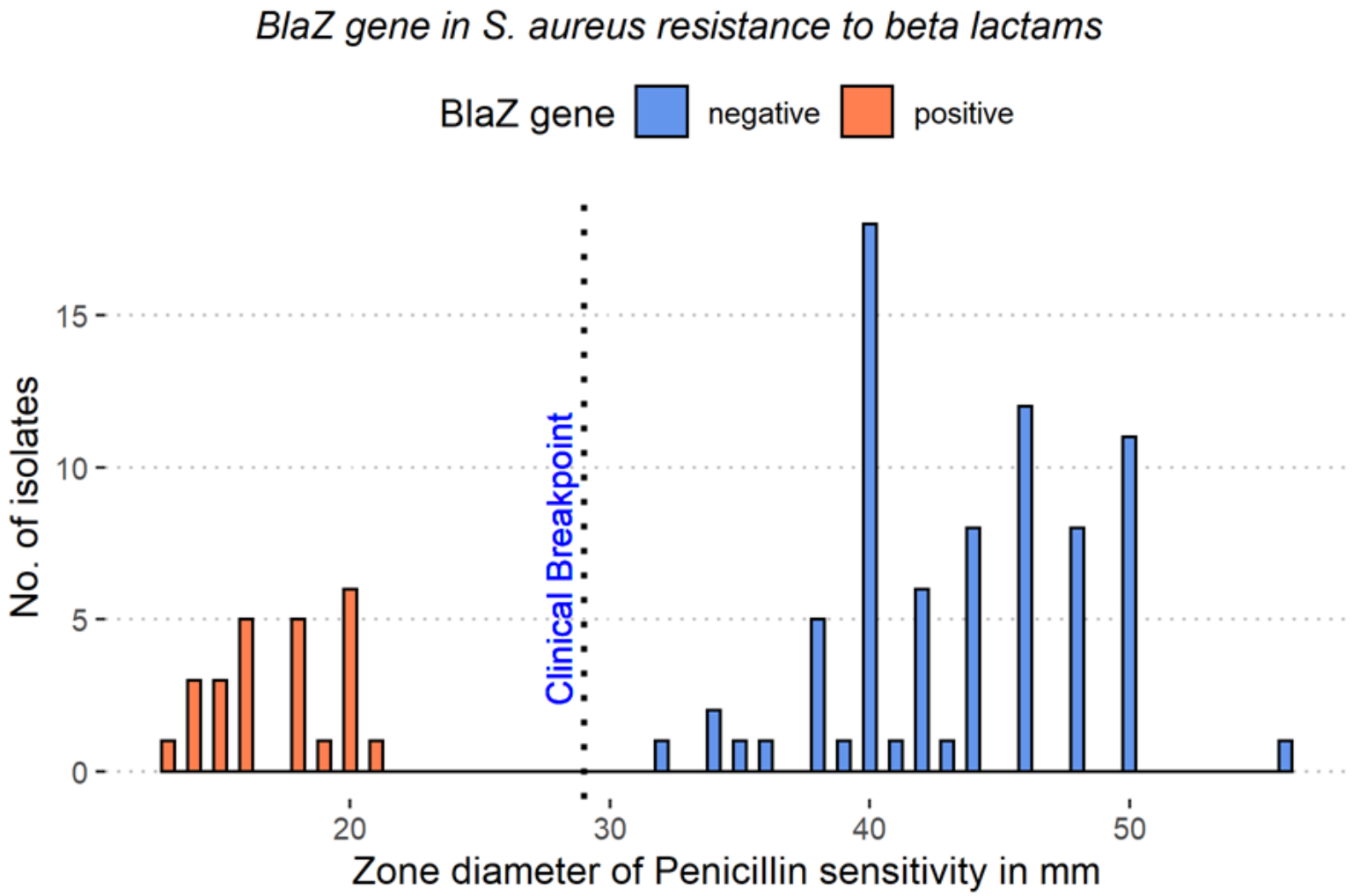


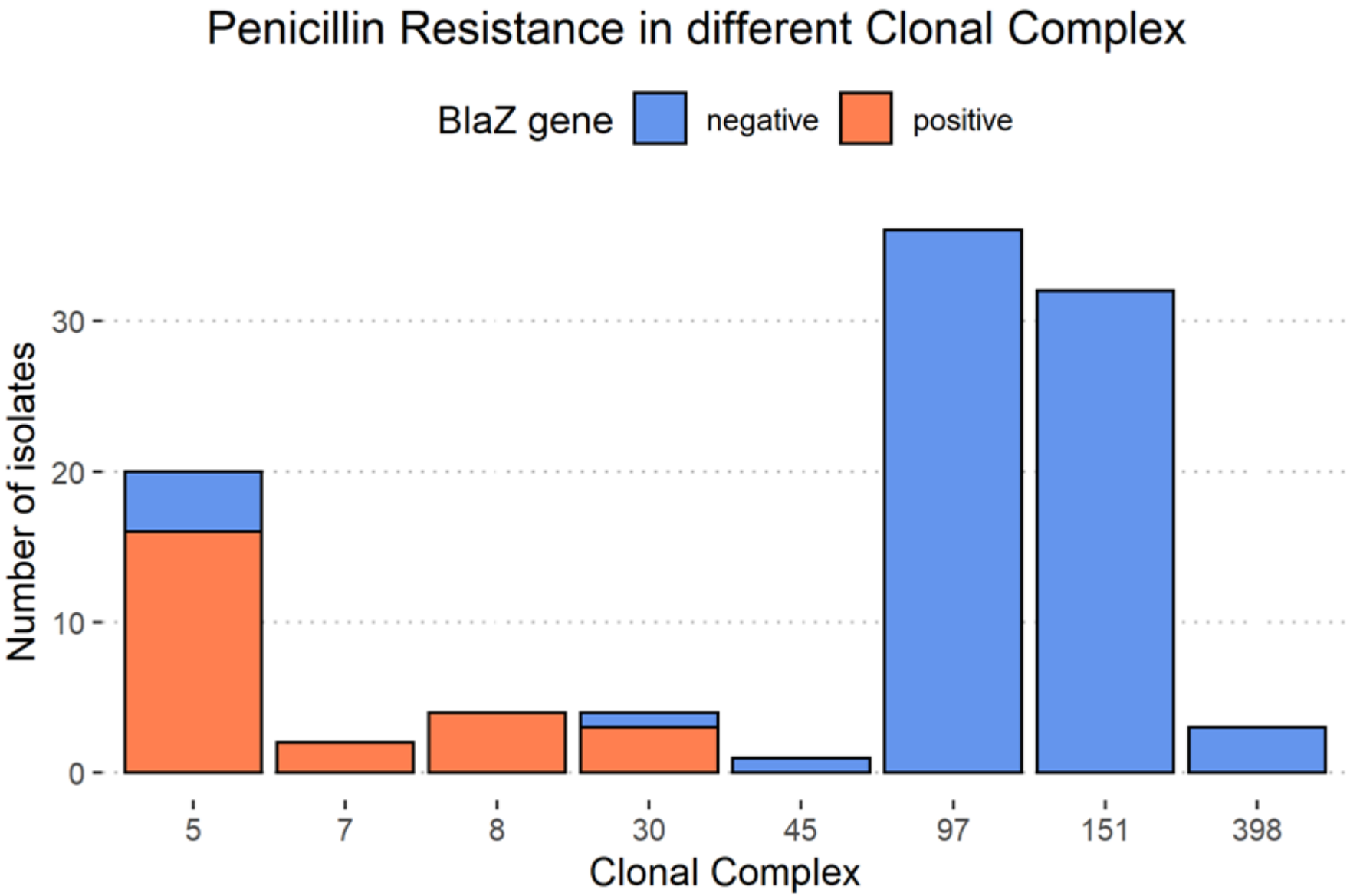
ST	CC	Source	Farm	Freq	Resist	Farm type
ST5957	CC5	H	3	7	100%	Org, Conv
ST5	CC5	H, B	4	11	63.6%	Org, Conv
ST148	CC5	B	1	1	100%	Org
ST1860	CC5	H	1	1	100%	Org
ST72	CC8	H	1	2	100%	Org
ST7	CC7	H	1	2	100%	Conv
ST8	CC8	H, C	2	2	100%	Conv
ST3028	CC97	B, C	6	17	0	Org, Conv
ST352	CC97	H, B, C	5	7	0	Conv
ST5958	CC97	C, B	1	9	0	Conv
ST3021	CC97	C, B	2	3	0	Conv
ST30	CC30	H	3	4	75%	Org, Conv
ST45	CC45	H	1	1	0	Org
ST398	CC398	H	1	3	0	Conv
ST2186	CC151	C	1	1	0	Conv
ST5956	CC151	C, B	1	3	0	Conv
ST151	CC151	C, B	9	28	0	Org, Conv

Resistance observed to

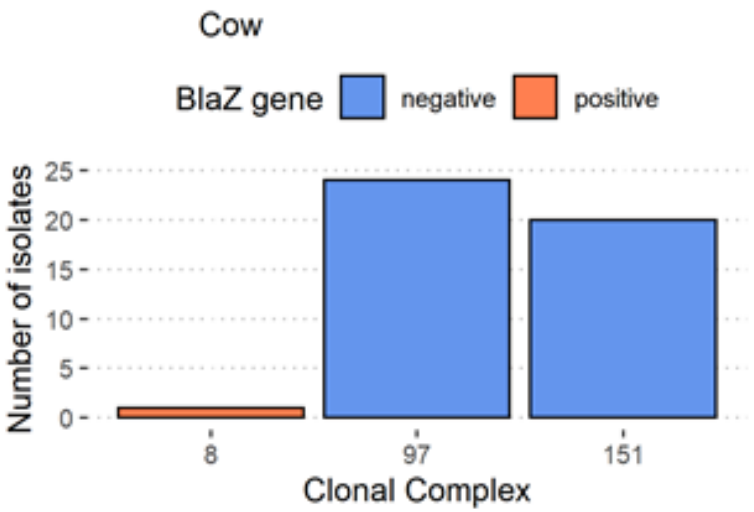
beta-lactams (Pen/Amp) 26%
tetracycline 5%
erythromycin 7%
lincomycin 3%
pirlimycin 2%
sulphadimethoxine 67%

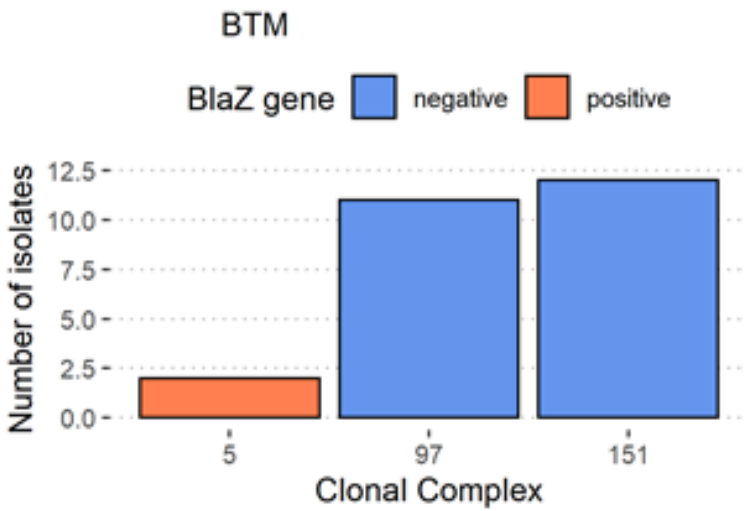


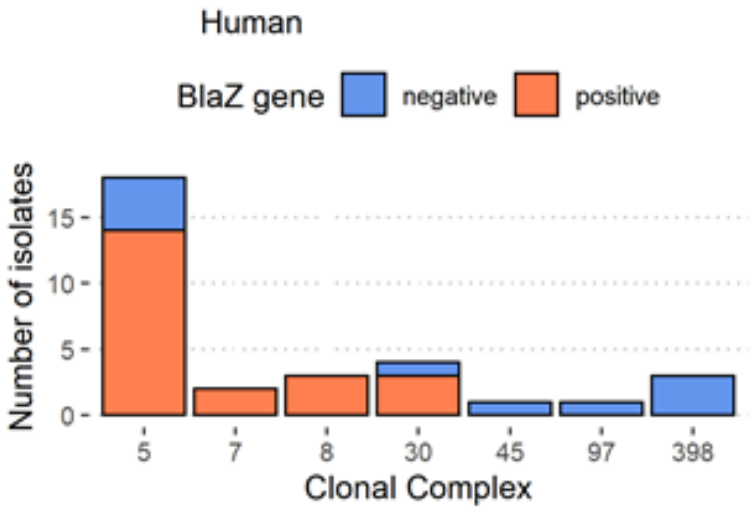



Penicillin Resistance in different Clonal Complex
BlaZ gene
negative
positive
Number of isolates
30
20
10
0
5
7
8
30
45
97
151
398
Clonal Complex

Penicillin resistant strains







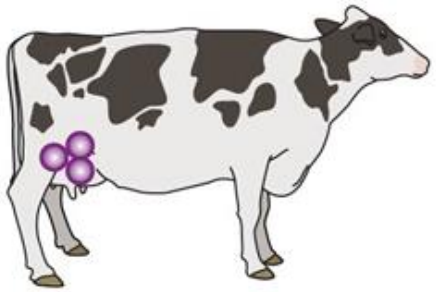


Table 1. Herd-level incidence rates for NASM IMI by facility type for 10 Vermont organic dairy herds

Bedded packs:

Herd	Incidence rate (cases/quarter-month)
1	0.0250
2	0.0067
3	0.0173
4	0.0273
5	0.0350

	Mean	***SE***	***95% CI***
Overall BP:	0.0222	0.0048	0.0089, 0.0356

Tiestalls:

Herd	Incidence rate (cases/quarter-month)
6	0.0070
7	0.0402
8	0.0166
9	0.0316
10	0.0360

	Mean	***SE***	***95% CI***
Overall TS:	0.0263	0.0063	0.0089, 0.0437
Total:	**0.0243**	**0.0038**	**0.0157, 0.0328**

~ 10 new NASM IMI per 100 cow-months / 10 new cases of subclinical IMI due to NASM each month on a 100-cow dairy

Incidence

Results: Incidence and elimination data sets

Complete cases overall		
	Number of complete cases	n = 1764 pairs of observations
	Number of quarters:	n = 1113 quarters
	Number of cows:	n = 343 different cows
	Number from visit 1/visit 2:	842 (47.7%)
	Number from visit 2/visit 3:	853 (48.4%)
	Number from visit 3/visit 4:	69 (3.9%)

Incidence data set		
	Number of pairs at risk for incident NASM IMI:	n = 1360 pairs of observations
	Number of quarters:	n = 899 quarters
	Number of cows:	n = 335 different cows

Elimination data set		
	Number of pairs at risk for eliminating NASM IMI:	n = 372 pairs of observations
	Number of quarters:	n = 230 quarters
	Number of cows:	n = 160 different cows

Table 3. Herd-level elimination rates for NASM IMI by facility type for 10 Vermont organic dairy herds

Bedded packs:

Herd	Elimination rate (cases/quarter-month)
1	0.0765
2	0.0900
3	0.0173
4	0.0525
5	0.1205

	Mean	***SE***	***95% CI***
Overall BP:	0.0714	0.0174	0.0230, 0.1197

Tiestalls:

Herd	Elimination rate (cases/quarter-month)
6	0.0429
7	0.1424
8	0.0178
9	0.0554
10	0.0959

	Mean	***SE***	***95% CI***
Overall TS:	0.0709	0.0219	0.0101, 0.1316
Total:	**0.0711**	**0.0132**	**0.0413, 0.1009**

~ 28 NASM IMI eliminated per 100 cow-months / 28 cases of subclinical IMI due to NASM eliminated each month on a 100-cow dairy

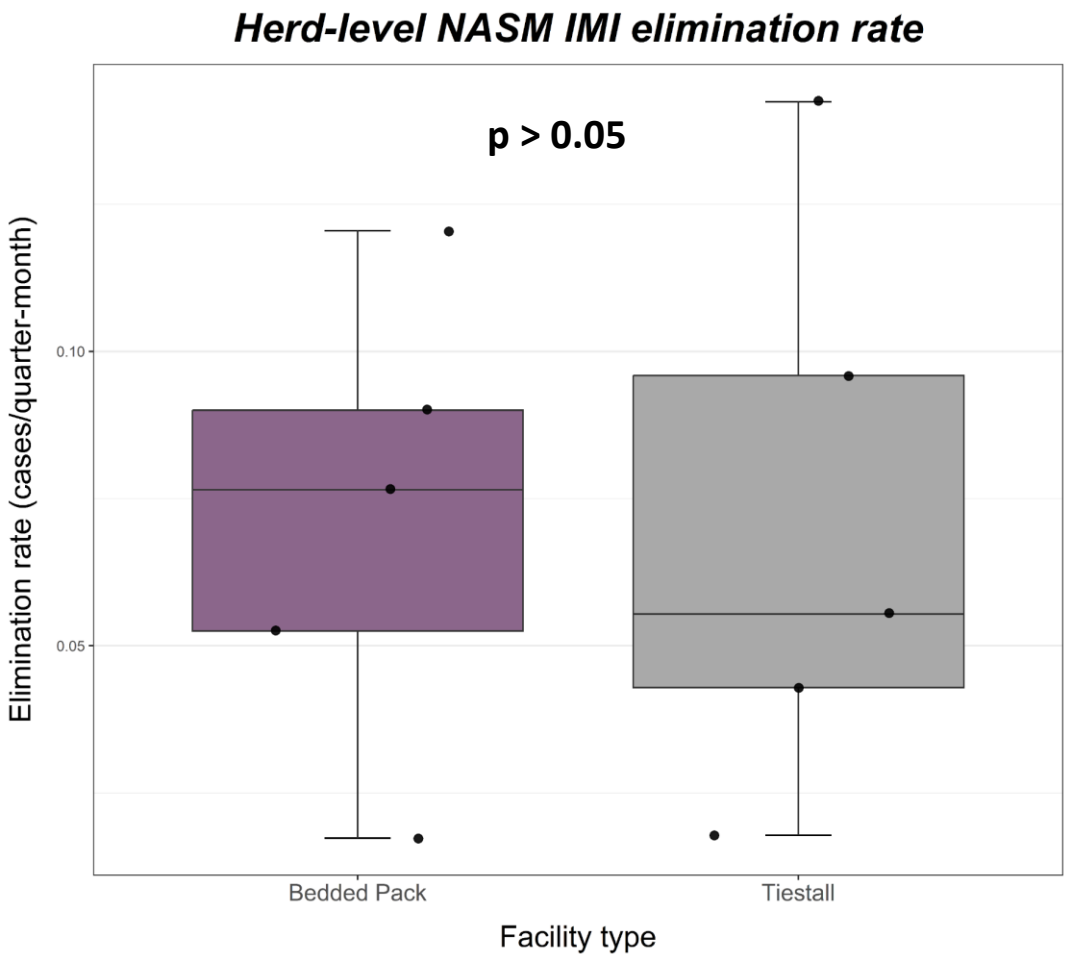


Table 4. Proportion of species causing eliminated NASM IMI on 10 Vermont organic dairy herds

NASM species	Number of eliminated IMI	Proportion of eliminated IMI
Staphylococcus chromogenes	11	37.93%
Staphylococcus equorum	7	24.14%
Staphylococcus haemolyticus	7	24.14%
Staphylococcus saprophyticus	1	3.45%
Staphylococcus simulans	1	3.45%
Staphylococcus succinus	1	3.45%
Staphylococcus xylosus	1	3.45%
Total	**29**	**100.00%**

Elimination

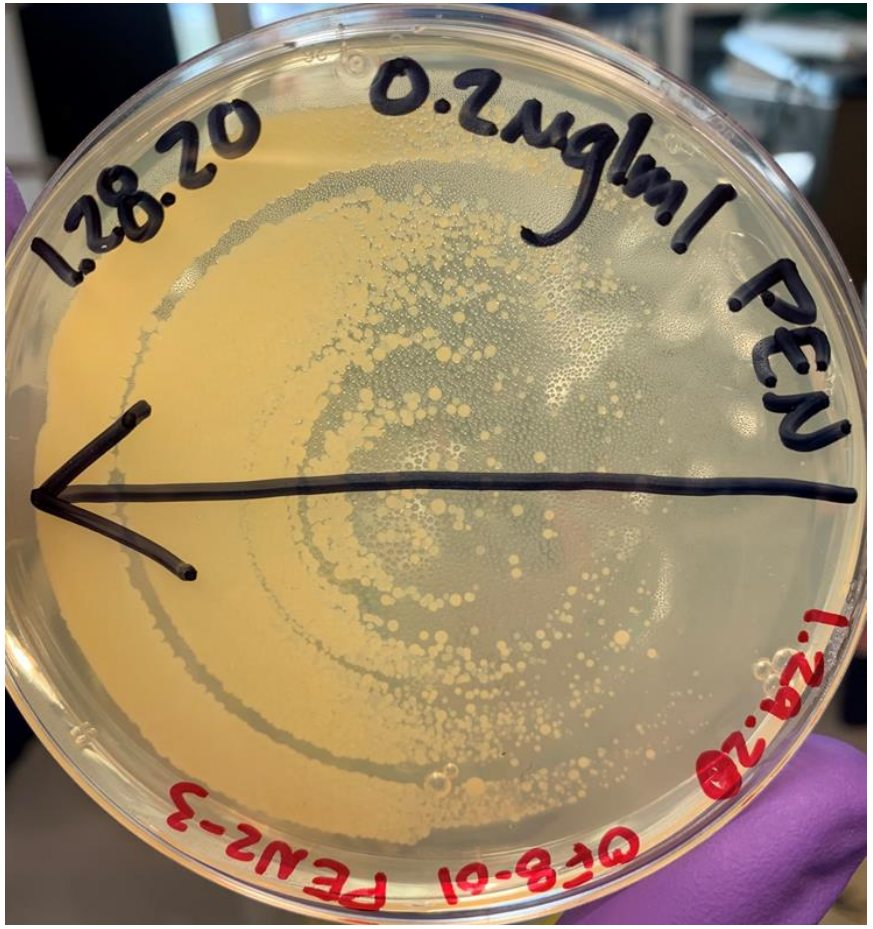
Observational study 2 – cross sectional survey

Table. Selected results of univariate analysis identifying factors unconditionally associated with udder health and hygiene outcomes at $p < 0.20$ for 21 Vermont organic dairy herds

Parameter	Group (sample size)	Coefficient estimate (SE)	*P*-value
Bulk tank milk somatic cell count (cells/mL)			
Lying surface	Mattress or concrete (n = 13)	36,000 (23,454)	0.14
	Deep bedding (n = 8)	*Ref.*	*Ref.*
Depth of bedding in stalls (cm)[1]	Tiestalls and freestalls (n = 15)	-5,797 (3,970)	0.17
Percent of cows with new IMI since last test date (%)[2]			
Glove use	Inconsistent glove use while milking (n = 9)	2.83% (1.7)	0.11
	All milkers consistently use gloves (n = 9)	*Ref.*	*Ref.*
Depth of bedding in stalls (cm)[1]	Tiestalls and freestalls (n = 15)	-0.62% (0.24)	0.02
Percent of cows with chronic IMI since last test date (%)[2]			
Herds that clip or flame udders one or more times per lactation	Yes (n = 5)	-4.31% (2.9)	0.16
	No (n = 14)	*Ref.*	*Ref.*
Proportion of cows with udder hygiene scores 3 + 4 (farm-level)	All herds with available test data (n = 19)	12.7% (6)	0.05
Average hygiene score (farm-level)	All herds with available test data (n = 19)	6.39% (3.1)	0.05
Percent of cows with SCC ≥ 200,000 cells/mL on current test date (%)[2]			
Depth of bedding in stalls (cm)[1]	Tiestalls and freestalls (n = 15)	-1.2% (0.42)	0.01
Proportion of cows with udder hygiene scores 3 + 4 (farm-level)	All herds with available test data (n = 19)	13.6% (8.5)	0.13
Average hygiene score (farm-level)	All herds with available test data (n = 19)	7.7% (4.3)	0.09
Average linear score of cows on farm (weighted)[3]			
Depth of bedding in stalls (cm)[1]	Tiestalls and freestalls (n = 15)	-0.06 (0.03)	0.05
Proportion of cows with udder hygiene scores 3 + 4 (farm-level)	All herds with available test data (n = 20)	0.85 (0.63)	0.2
Average hygiene score (farm-level)			
Depth of bedded pack (m)	Bedded pack herds (n = 5)	-0.96 (0.15)	< 0.01
Lying surface	Mattress or concrete (n = 13)	0.33 (0.16)	0.06
	Deep bedding (n = 8)	*Ref.*	*Ref.*
Depth of bedding in stalls (cm)[1]	Tiestalls and freestalls (n = 15)	-0.06 (0.03)	0.07

[1] Stall bedding depth for freestalls and tiestalls bedded with wood shavings or sawdust
[2] DHIA data available for n = 19 herds. One herd included in average linear score analyses is seasonal and had no recent test data.
[3] DHIA data available for n = 20 herds.

Serial Antimicrobial Exposure & Selecting for Changes in Antimicrobial Susceptibility



(a)

(b)

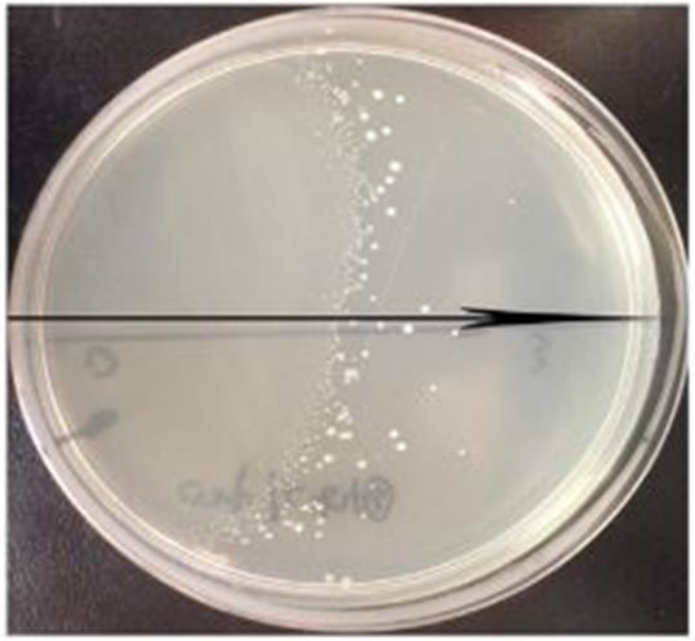
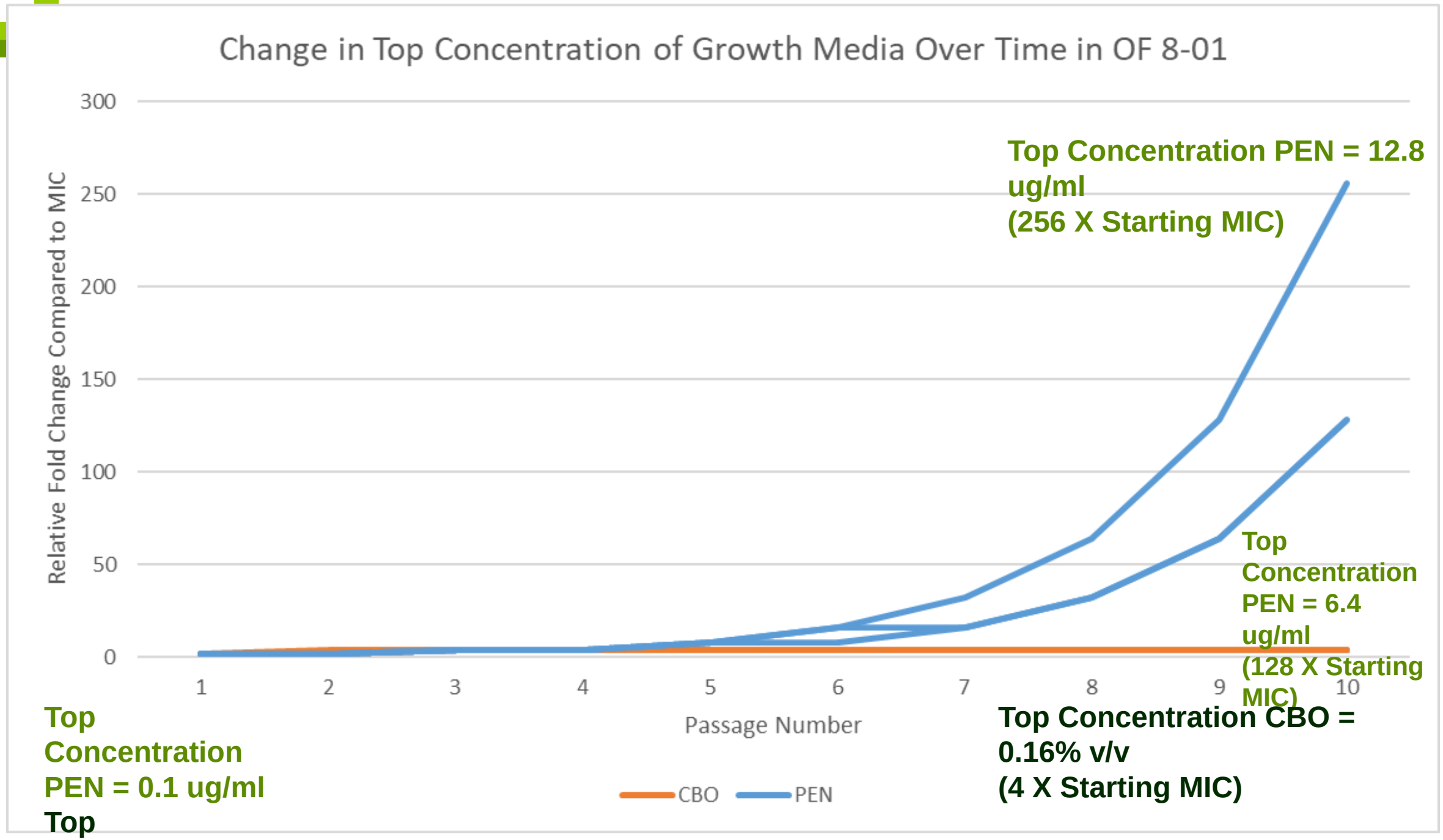


Fig. 1. Gradient plate of two agar layers.


Change in Top Concentration of Growth Media Over Time in OF 8-01
Relative Fold Change Compared to MIC
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
Passage Number
CBO
PEN
Top Concentration PEN = 12.8 ug/ml (256 X Starting MIC)
Top Concentration PEN = 6.4 ug/ml (128 X Starting MIC)
Top Concentration PEN = 0.1 ug/ml
Top Concentration CBO = 0.16% v/v (4 X Starting MIC)
Top Concentration