Final report

Effect of ionizing radiation on important foodborne bacteria during meat processing

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Abstract

The Australian meat and livestock industry utilises X-ray radiation technology to assist in the grading of beef or lamb carcasses, and to drive automated cutting and deboning. One system supported by Meat & Livestock Australia for objective measurement of carcases uses Dual Energy X-ray Absorptiometry (DEXA). This system has a radiation load which is similar to regular X-rays, although less than that produces by computed tomography (CT) scans used in human medicine. One concern with the routine use of this technology in the meat industry is the potential for radiation to cause mutations of remnant carcase contaminating bacteria, leading to heritable genotypic and phenotypic changes in these bacterial populations. Given the meat industry's commitment to public health and quality assurance, and to eliminate community concern, it is important to conduct a robust experimental assessment of the risk of industry level radiation causing such changes in carcase contaminating bacteria. This study addresses the potential for ionizing radiation from meat imaging and sterilization to cause heritable genotypic and phenotypic changes in carcase contaminating bacteria, and whether these changes would constitute a public health concern.

There were three phases in this study: a field-based assessment, a culture laboratory-based assessment, and a product-based assessment. Each phase involved exposing bacteria to irradiation, performing minimum inhibitory concentrations (MICs) against a panel of antimicrobials, and determining if there were significant phenotypic and genetic changes in these bacteria.

The data from this study suggests that ionizing radiation from meat imaging is unlikely to cause heritable genotypic and phenotypic changes in carcass contaminating bacteria. Whilst there were some MIC changes, this can be attributed to assay variations.

Executive summary

Background

The Australian meat and livestock industry utilise X-ray radiation technology to assist in the grading of beef or lamb carcasses, and to drive automated cutting and deboning. Called objective carcass measurement technology, the improvement is believed to add $400M annually to the industry by providing transparent and scientific measurements of meat quality to buyers and creating valuable feedback to farmers.

One system currently on trial by Meat & livestock Australia for objective measurement of carcases uses Dual Energy X-ray Absorptiometry (DEXA), which is similar to regular X-rays and computed tomography (CT) scans used in human medicine. Other than meat grading, powerful X-rays and Gamma rays have been employed to sterilize meat and other fresh produce to reduce or eliminate harmful bacteria, therefore rendering it safer and extending its shelf-life. One concern with the routine use of this technology in the meat industry is the potential for radiation to cause mutations of remnant carcase contaminating bacteria, leading to heritable genotypic and phenotypic changes in these bacterial population.

Given the meat industry's commitment to public health and quality assurance, and to eliminate community concern, it is important to conduct a robust experimental assessment of the risk of industry level radiation causing such changes in carcase contaminating bacteria. To address this, this study explored the possibility of ionizing radiation from meat imaging and sterilization to cause heritable genotypic and phenotypic changes in carcase contaminating bacteria, and whether or not potential changes would constitute a public health concern.

Objectives

1. Determine the development of radio-resistance of bacteria found on meat carcasses, including what level of radiation or repeat dosing can be tolerated before mutations occur.
2. Determine the impact of radio imaging and sterilisation on bacteria DNA with regards to resistance development against disinfectants and/or antimicrobials.

Methodology

This study comprised of three phases, a field-based assessment, a culture laboratory-based assessment and a product-based assessment.

Results/key findings

The key findings from this study suggests that ionizing radiation from meat imaging is unlikely to cause heritable genotypic and phenotypic changes in carcass contaminating bacteria.

Benefits to industry

Given the meat industry's commitment to public health and to address potential public health concern surrounding X-ray radiation technology, the results from this study are crucial to determine whether ionising radiation of meat carcasses cause heritable genotypic and phenotypic changes in carcase contaminating bacteria.

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1. Background

The Australian meat and livestock industry utilise X-ray radiation technology to assist in the grading of beef or lamb carcasses, and to drive automated cutting and deboning. Called objective carcass measurement technology, the improvement is believed to add $400M annually to the industry by providing transparent and scientific measurements of meat quality to buyers and creating valuable feedback to farmers.

One system currently supported by Meat & livestock Australia for objective measurement of carcases uses Dual Energy X-ray Absorptiometry (DEXA) which is similar to regular X-rays and computed tomography (CT) scans used in human medicine. Other than meat grading, powerful X-rays and Gamma rays are employed to sterilize meat and other fresh produce to reduce or eliminate harmful bacteria, therefore rendering it safer and extending its shelf-life. One concern with the routine use of this technology in the meat industry is the potential for radiation to cause mutations of remnant carcase contaminating bacteria, leading to heritable genotypic and phenotypic changes in these bacterial population.

Given the meat industry's commitment to public health and quality assurance, and to address potential community concern, it is important to conduct a robust experimental assessment of the risk of industry level radiation causing such changes in carcase contaminating bacteria. To address this, this study will explore the possibility of ionizing radiation from meat imaging and sterilization to cause heritable genotypic and phenotypic changes in carcase contaminating bacteria, and whether or not potential changes would constitute a public health concern.

2. Objectives

1. Determine the development of radio-resistance of bacteria found on meat carcasses, including what level of radiation or repeat dosing can be tolerated before mutations occur.
2. Determine the impact of radio imaging and sterilisation on bacteria DNA with regards to resistance development against disinfectants and/or antimicrobials.

3. Methodology

3.1 Phase 1 Field-based assessment

Experimental plan

- Lamb carcass swabs were taken from commercial abattoir pre-DEXA and post-DEXA exposure for recovery of three indicator bacterial genera (*E. coli*, *Enterococci*, *Aeromonas etc*)
- Swabs were subsequently plated onto sheep blood agar plates using standard procedures for evaluation of aerobic bacteria
- Blood agar plates were imaged to determine if DEXA has any effect on increasing or decreasing on-carcass bacterial load
- Representative bacterial colonies were identified by MALDI-TOF (BRUKER)
- Representative bacteria were selected for minimum inhibitory concentration (MIC) testing by ISO9001 CLSI antimicrobial susceptibility testing and subjected to DNA sequencing if any differences in antimicrobial resistance are identified

3.2 Phase 2 Culture laboratory-based assessment

Experimental plan

- 20 carcass swabs were taken from a commercial abattoir and submitted to Murdoch University AMRID laboratory.
- The bacteria from the swabs were cultured and isolated on sheep blood agar with species identification performed using MALDI-TOF (Figure 1).
- Generic bacteria species were selected for irradiation using an in-house CT scanner. Three ATCC control strains for *E. faecalis*, *S. aureus* and *E. coli* were also included in the experiment.
- Five replicate copies of each bacterial species were sub-cultured onto sheep blood agar and irradiated at four different power settings. The remaining unirradiated bacteria were used as experimental control.
- Immediately after irradiation, MICs were performed for bacteria from the highest irradiation setting and the experimental control plate (Figure 2).
- Isolates demonstrating any discrepancies in were subjected to further testing using samples subjected to lower-level irradiation.
- Whole genome sequencing was performed for isolates displaying a change in MIC values between control and test isolates.

3.3 Phase 3 Product-based laboratory assessment

Experimental plan

- Bacterial species used in Phase 2 were inoculated onto lamb steaks and exposed to the highest level of irradiation (135 kV) at Murdoch University which is comparable to DEXA.
- Bacteria and ATCC control strains were sub-cultured onto sheep blood agar.
- A known concentration of bacteria (10^5 CFU/10 CM^2) was inoculated onto lamb samples and each set of samples were exposed to radiation at 135 kV.
- Bacteria was recovered from pre-irradiation samples and plated onto sheep blood agar.
- Eight colonies of the irradiation samples (test) were selected from the agar plates and species confirmed on the MALDI-TOF.
- MIC testing was performed on isolates with no irradiation (control) and bacterial isolates exposed to the highest levels of irradiation (test).
- Whole genome sequencing was performed for isolates displaying a change in MIC values (>2 dilution) between control and test isolates.

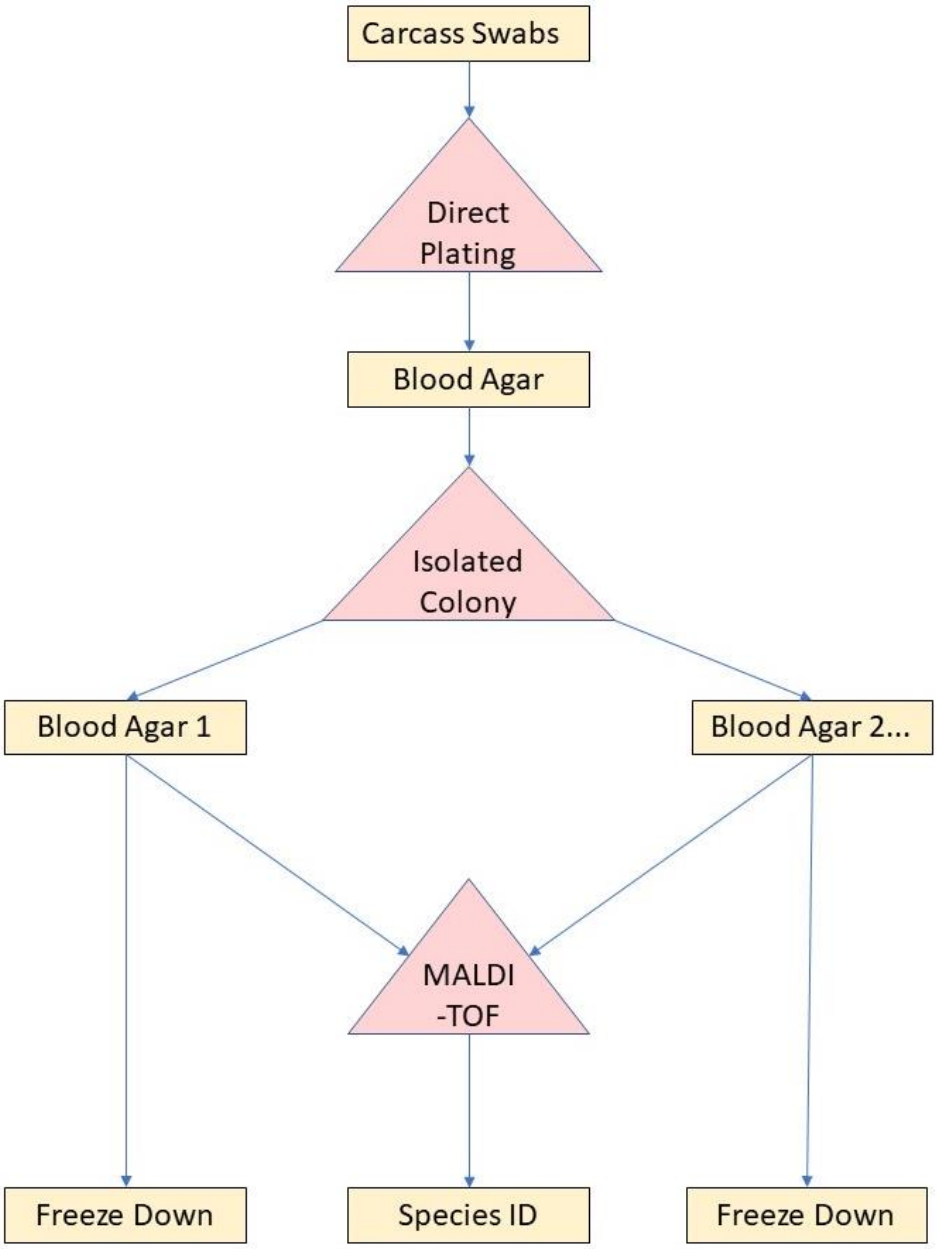


Figure 1: Outline of methods for carcass swab isolates

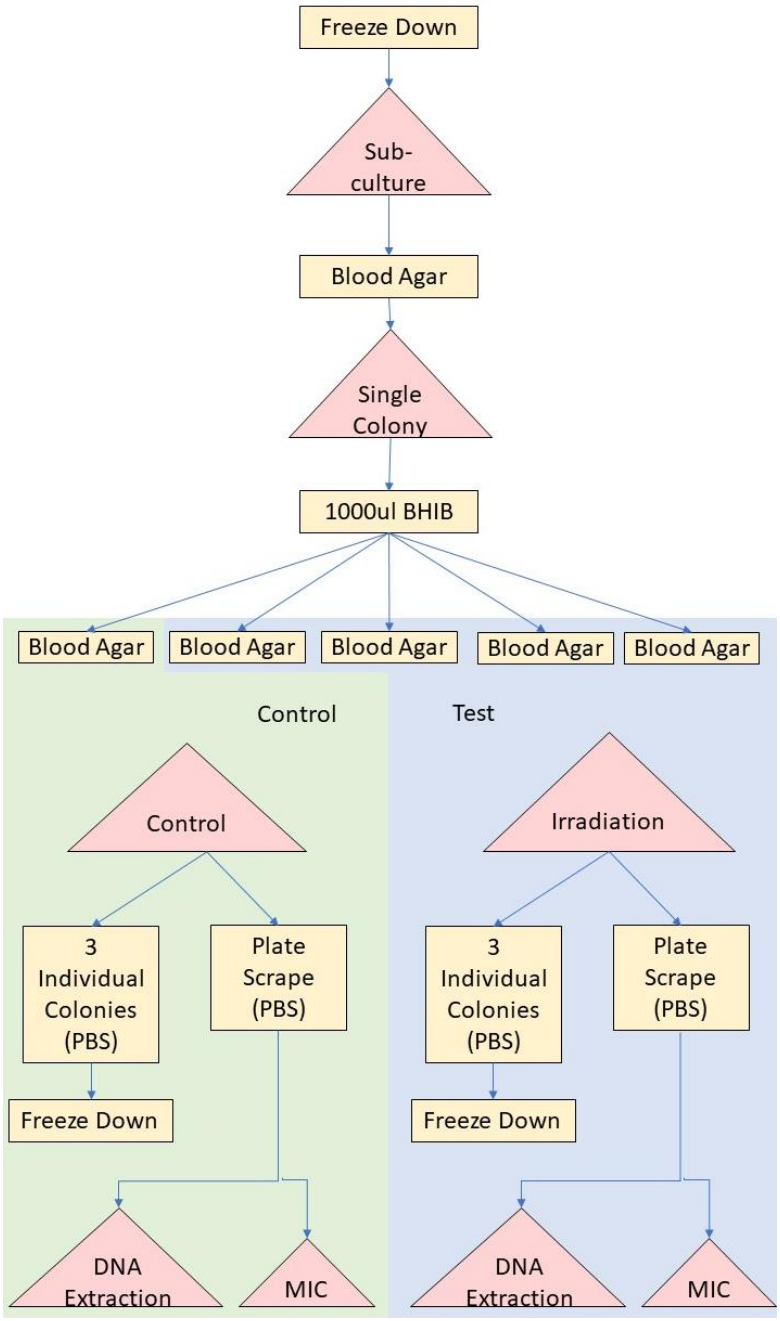


Figure 2: Process for performing MIC testing on cultures isolated as per Figure 1

4. Results

4.1 Phase 1 Field based assessment

A total of 17 lamb carcasses were swabbed pre- and post-DEXA exposure. Swabs were taken from the left (pre-DEXA) or right (post-DEXA) of lamb leg. Swabs were plated onto sheep blood agar and incubated overnight. Images of each individual plate were taken (Appendix 7.1) and representative bacterial colonies were identified by MALDI-TOF.

A summary of the bacteria isolated and identified by MALDI-TOF from each carcass swab can be found in Appendix 7.2. Species isolated from both before and after swabs include *Escherichia coli*, *Acinetobacter* spp., *Enterococcus* spp., *Staphylococcus* spp., *Glutamicibacter* spp., *Desemzia incerta*, *Psychrobacter pulmonis*, *Aerococcus viridans* and *Ignatzschineria* spp. These bacteria were selected for minimum inhibitory concentration (MIC) testing by ISO9001 CLSI antimicrobial susceptibility testing. Some bacterial species such as *Moraxella osloensis*, *Exiguobacterium mexicanum*, *Aeromonas hydrophila*, *Bacillus licheniformis* and *Solibacillus silvestris* were only isolated from before or after swabs and were not included for MIC testing as a comparative MIC analysis would not be possible.

MIC results can be found in Appendix 7.3. Isolates that did not yielded growth over-night as per CLSI recommended assay conditions were removed from down-stream analysis. This included all *Desemzia incerta* isolates and one *Acinetobacter parvus* isolate which showed no growth in the growth control during MIC testing and was removed from MIC analysis. As per CLSI guidelines, for *Enterococcus* spp., cephalosporins and trimethoprim-sulfamethoxazole may appear active *in vitro*, but they are not effective clinically. As such, cefotaxime, ceftazidime, trimethoprim and sulfamethoxazole *Enterococcus* results were not reported for MIC analysis. One *Psychrobacter pulmonis* isolate (22050030_02) showed a higher sulfamethoxazole result (before DEXA), however none of the post DEXA isolates showed a high sulfamethoxazole MIC result. Overall, the MIC values for before and after isolates were within a similar dilution range, and there were no discernible changes in MIC values (i.e. MIC value significantly higher in isolate after DEXA irradiation).

4.2 Phase 2 Culture laboratory-based assessment

From 20 carcass swabs submitted for processing at the AMRID laboratory, a total of 48 bacterial isolates were collected. MALDI-TOF MS was performed on all isolates returning 19 unique species of bacteria. 10 of the 19 isolates and 3 ATCC control strains were selected based on their public health risk and known prevalence for AMR for further testing.

A single colony of each bacterium was sub-cultured onto five sheep blood agars and incubated overnight at 37°C. One copy of each bacterium was set aside as control while four test copies were subjected to X-ray radiation via a CT scanner at varying intensities of 80, 100, 120 and 135 kV @ 123 mAs. Each test set was given an ID accordingly as test80, test100, test120 and test135. After irradiation, the control and test135 isolates were subjected to MIC test using the broth microdilution method on a six-drug panel (ampicillin, tetracycline, ciprofloxacin, gentamycin, ceftriaxone and trimethoprim-sulfamethoxazole) and whole genome sequencing.

A total of 11 MIC result discrepancies were identified between control and test135 isolates. However, only one discrepancy had greater than 1 dilution difference (Table 1). As per CLSI guidelines, for *Enterococcus* spp., cephalosporins and trimethoprim-sulfamethoxazole may appear active *in vitro*, but they are not effective clinically. As such, ceftriaxone, trimethoprim and

sulfamethoxazole *Enterococcus* results were not reported for MIC analysis. All control and test135 isolates were sequenced using the Illumina NextSeq 550 platform. When control and corresponding test isolates were compared, a minimum of 0 to a maximum of 130 mutations were identified which includes complex and single nucleotide mutations. None of the mutations were located in genes responsible for antimicrobial resistance. Please note, assay variation is very common while performing MIC testing and a MIC elevation greater than two-fold dilution is considered significant.

4.3 Phase 3 Product-based laboratory assessment

Bacterial species used in Phase 2 were inoculated onto lamb steaks and exposed to the highest level of irradiation (135 kV) which is comparable to DEXA. Bacteria was recovered from the lamb samples and eight colonies were picked and MICs at the pre (control) and post (test) isolates were determined (Appendix 7.4). MIC results showed that majority of the MIC value for control and test isolates were the same. As per CLSI guidelines, for *Enterococcus* spp., cephalosporins and trimethoprim-sulfamethoxazole may appear active *in vitro*, but they are not effective clinically. As such, cefotaxime, ceftazidime, trimethoprim and sulfamethoxazole *Enterococcus* results were not reported for MIC analysis. Three isolates showed MIC higher than the control (un-exposed isolate). These isolates were *E. coli* 402 Test 4, 5 and 6 MIC results where there were two dilutions higher than control for gentamicin. *E. coli* 402 control, Test 4, 5 and 6 were subsequently sequenced using Illumina NextSeq 550 platform. When control and corresponding test isolates were compared, two mutations were located in genes responsible for antimicrobial resistance on both the control and test isolates (Table 3) indicating that the mutations were already there before irradiation and not attributed to irradiation. The reported MIC values are within the expected QC ranges recommended clinical laboratory standards institute guidelines. In addition, elevation of MIC drift for these antimicrobials has been reported previously and is likely due to point mutations or assay variations (Truswell et al. 2021).

Table 1: Minimum inhibitory concentration results for control (not irradiated) and isolates irradiated at 135 kV (test135 isolates)

*NR = not reported

		Control						Test135 isolates					
ID	MALDI Species	AMP	TET	CIP	GENT	CEFT	SXT	AMP	TET	CIP	GENT	CEFT	SXT
01-02	*E. hirae*	<1	<0.5	<0.015	2	NR	NR	1	0.5	<0.015	2	NR	NR
01-04	*C. divergens*	16	2	0.06	<0.25	<0.03	0.25	16	2	0.06	<0.25	<0.03	0.25
04-02	*E. coli*	16	2	0.015	<0.25	<0.03	0.5	16	2	0.03	<0.25	<0.03	0.25
05-01	*A. guilloulae*	2	<0.5	0.06	<0.25	2	0.25	2	<0.5	0.06	<0.25	2	0
05-03	*S. saprophyticus*	<1	1	0.125	<0.25	4	0.25	<1	1	0.125	<0.25	>4	0.25
06-02	*K. pneumoniae*	128	4	0.03	<0.25	>4	0.25	128	4	<0.015	<0.25	>4	0.25
10-03	*B. pumilus*	8	1	0.125	<0.25	>4	0.25	4	1	0.25	<0.25	>4	0.25
13-02	*E. coli*	8	1	<0.015	<0.25	<0.03	0.25	8	1	<0.015	<0.25	<0.03	0.25
14-01	*S. equorum*	<1	1	0.125	<0.25	4	0.25	<1	2	0.125	<0.25	4	0.25
14-02	*A. baumanii*	64	4	0.125	0.5	4	0.25	64	4	0.06	<0.25	4	0.25
ATCC25922	*E. coli*	32	2	<0.015	<0.25	0.06	0.25	32	2	<0.015	<0.25	0.06	0.25
ATCC25923	*S. aureus*	<1	<0.5	0.25	<0.25	4	0.25	<1	1	0.25	<0.25	4	0.25
ATCC29212	*E. faecalis*	8	32	0.25	4	NR	NR	8	32	0.25	4	NR	NR

Table 3: Sequencing data for isolates which showed two dilutions higher than control. glpT_E448K and uhpT_E350Q refer to point mutations

Isolate	Species	Genotype	glpT_E448K	uhpT_E350Q
402E-C	*Escherichia coli*	*acrF,blaEC,glpT_E448K,mdtM,uhpT_E350Q*	1	1
402E-T4	*Escherichia coli*	*acrF,blaEC,glpT_E448K,mdtM,uhpT_E350Q*	1	1
402E-T5	*Escherichia coli*	*acrF,blaEC,glpT_E448K,mdtM,uhpT_E350Q*	1	1
402E-T6	*Escherichia coli*	*acrF,blaEC,glpT_E448K,mdtM,uhpT_E350Q*	1	1

5. Conclusion

The experimental data from Phase 1, Phase 2 and Phase 3 suggest that ionising radiation is unlikely to result in heritable changes to commensal bacteria commonly found on meat or meat processing environment. It should be noted that bacteria do mutate naturally by point mutations that may result in elevation of MICs when tested in laboratory conditions. We were unable to determine if DEXA had any effect on increasing or decreasing on-carcass bacterial load as it appears that this is dependent on multiple factors in the abattoir such as the initial bacterial load, distribution on the carcass, the actual handling of the carcass, and potential surface contamination.

5.1 Key findings

The data from this study suggests that ionizing radiation from meat imaging is unlikely to cause heritable genotypic and phenotypic changes in carcass contaminating bacteria.

5.2 Benefits to industry

Given the meat industry's commitment to public health and to manage potential public health concern surrounding X-ray radiation technology, results from this study are crucial to determine whether ionising radiation of meat carcasses cause heritable genotypic and phenotypic changes in carcase contaminating bacteria.

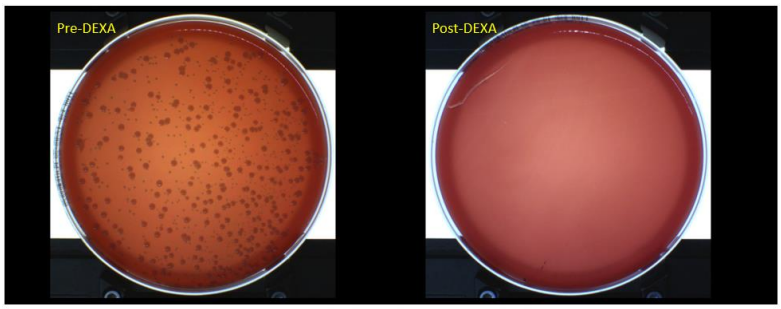
6. References

Truswell, A., R. Abraham, M. O'Dea, Z. Z. Lee, T. Lee, T. Laird, J. Blinco, S. Kaplan, J. Turnidge, D. J. Trott, D. Jordan, and S. Abraham. 2021. 'Robotic Antimicrobial Susceptibility Platform (RASP): a next-generation approach to One Health surveillance of antimicrobial resistance', *J Antimicrob Chemother*, 76: 1800-07.

7. Appendix

7.1 Phase 1 Field based assessment – plate images

Carcass 1



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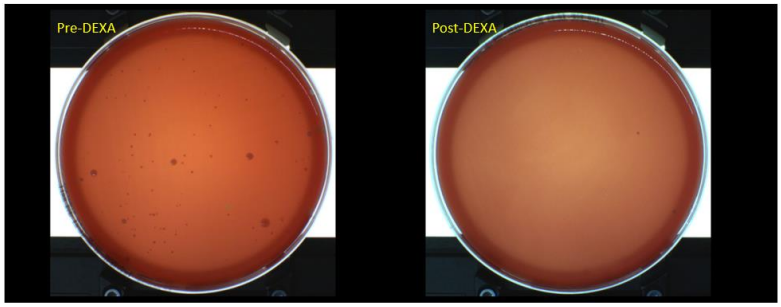

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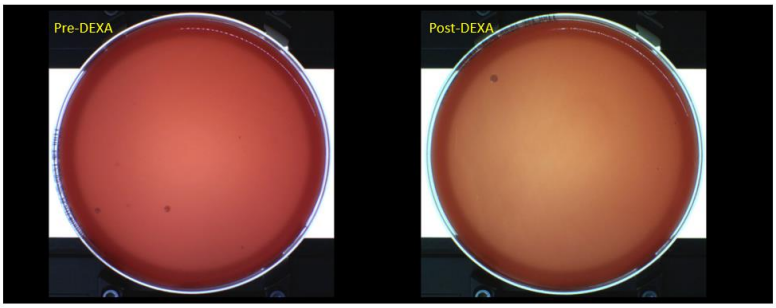

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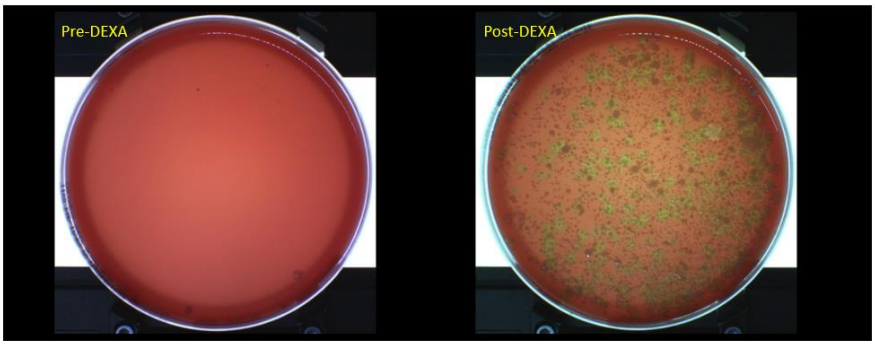

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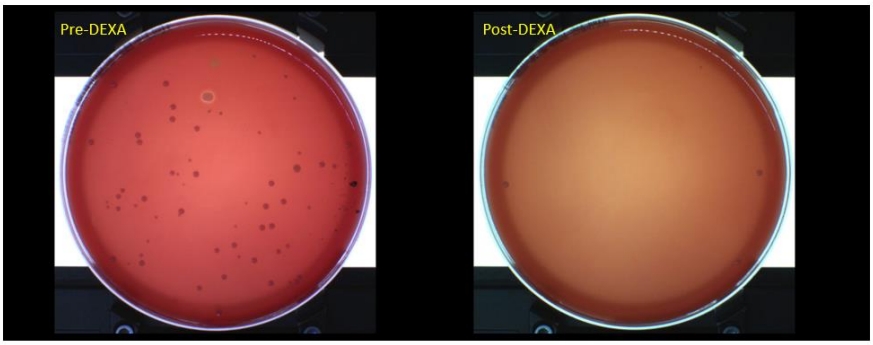

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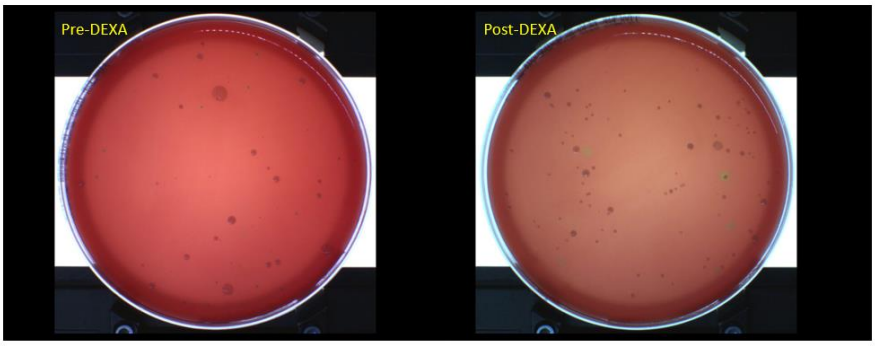

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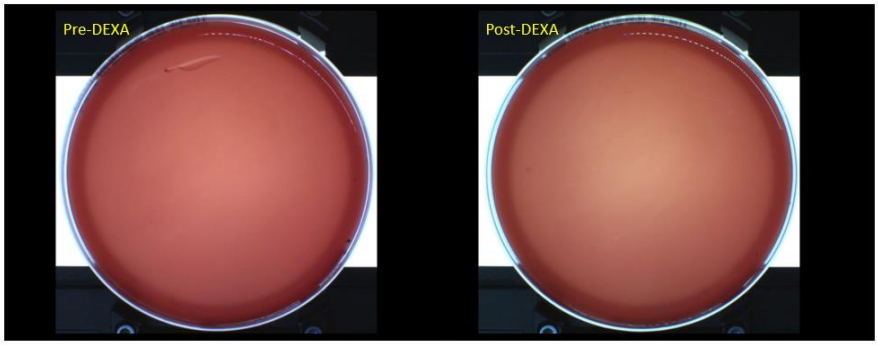

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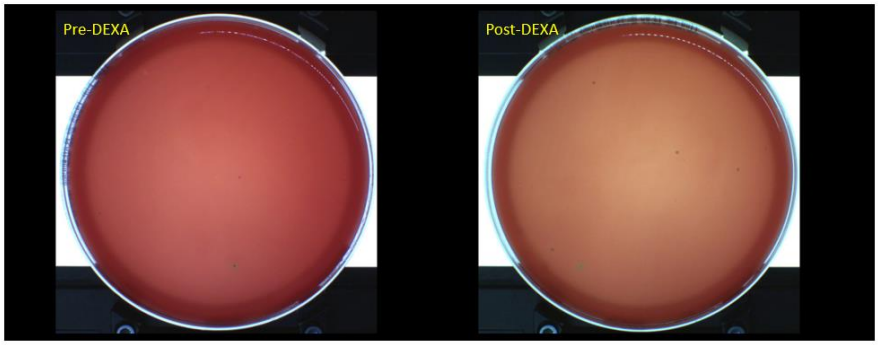

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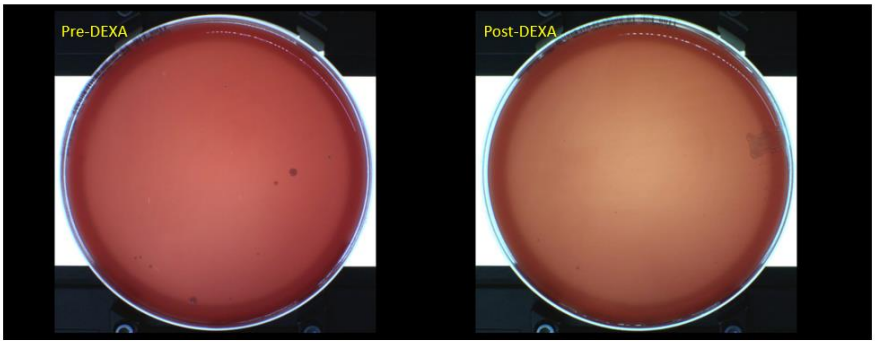

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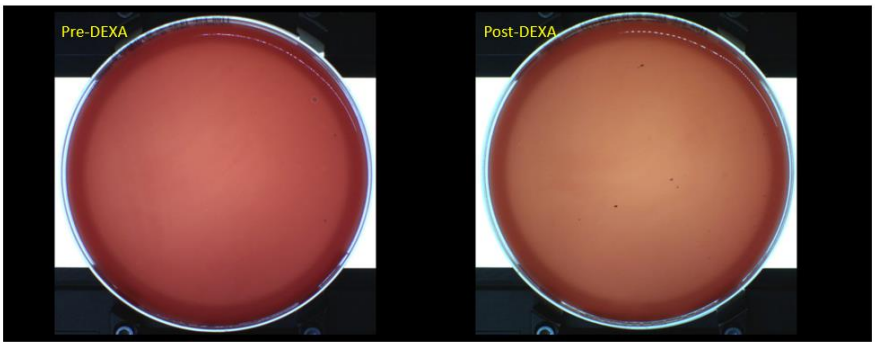

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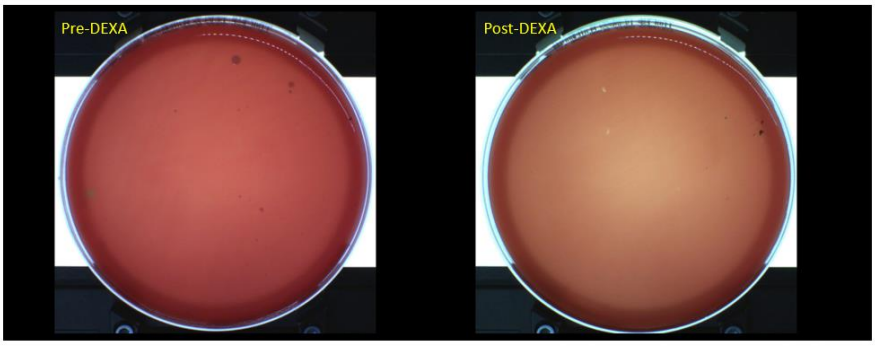

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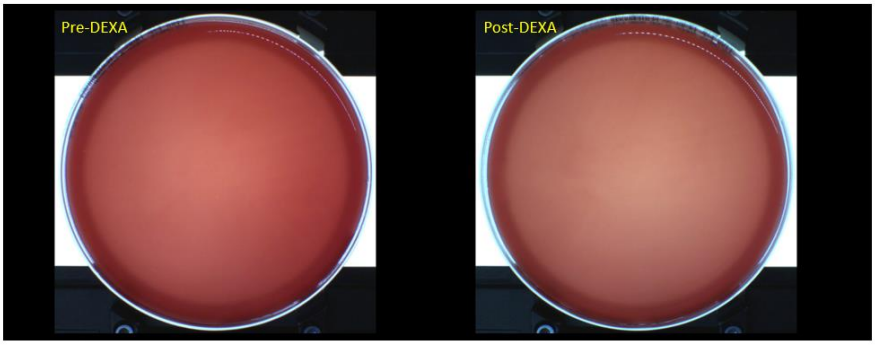

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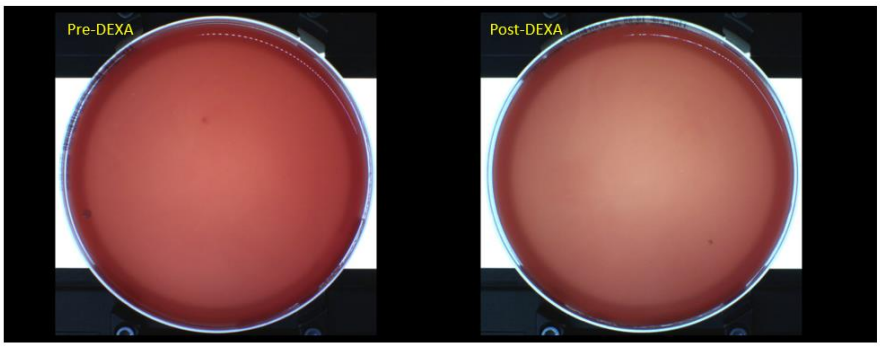

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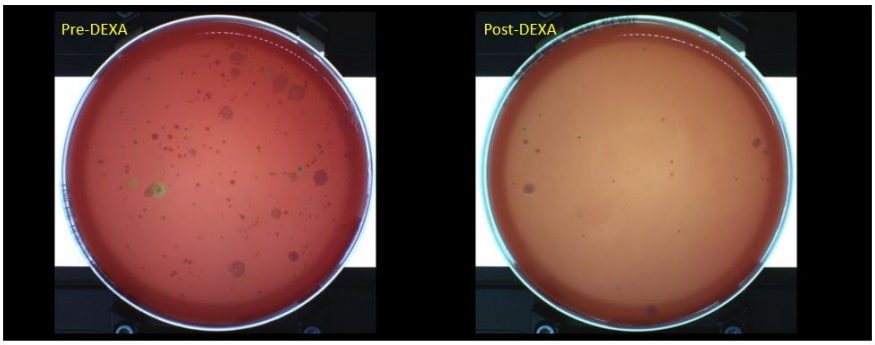

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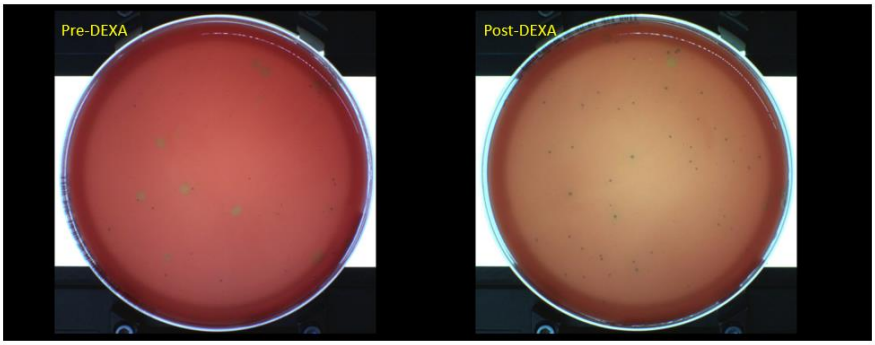

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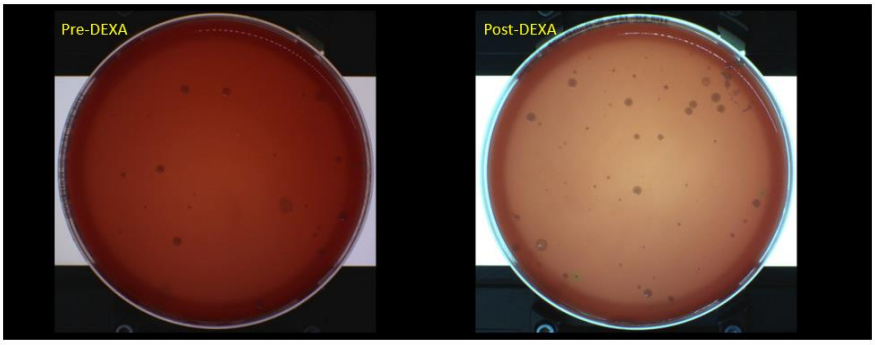

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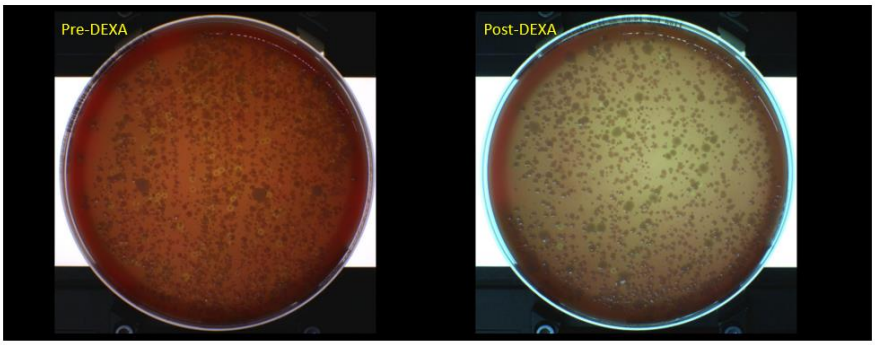

7.2 Phase 1 Field based assessment – bacteria isolated from carcasses

A= after DEXA B=before DEXA	**Bacteria**	**Unique ID**
A1	No growth	No growth
A2	*Enterococcus gallinarium*	22050002
A3	*Escherichia coli*	22050003
A4	*Acinetobacter lwoffii*	22050004_01
A4	*Staphylococcus equorum*	22050004_02
A5	*Acinetobacter lwoffii*	22050005
A6	*Desemzia incerta*	22050006_01
A6	*Acinetobacter pseudolwoffii*	22050006_02
A7	*Acinetobacter schindleri*	22050007
A8	*Enterococcus gallinarium*	22050008
A9	*Morexella osloensis*	22050009
A10	No growth	No growth
A11	*Psychrobacter pulmonis*	22050011_01
A11	*Enterococcus casseliflavus*	22050011_02
A11	*Staphylococcus equorum*	22050011_03
A12	No growth	No growth
A13	*Psychrobacter pulmonis*	22050013_01
A13	*Acinetobacter lwoffii*	22050013_02
A14	*Psychrobacter pulmonis*	22050014_01
A14	*Aerococcus viridans*	22050014_02
A14	*Escherichia coli*	22050014_03
A14	*Glutamicibacter arilaitensis*	22050014_04
A15	*Ignatzschineria ureiclastica*	22050015_01
A15	*Glutamicibacter protophormiae*	22050015_02
A16	*Exiguobacterium mexicanum*	22050016_01
A16	*Acinetobacter lwoffii*	22050016_02
A16	*Desemzia incerta*	22050016_03
A17	*Escherichia coli*	22050017_01
A17	*Ignatzschineria indica*	22050017_02
B1	*Escherichia coli*	22050018_01
B1	*Enterococcus gallinarium*	22050018_02
B2	*Staphylococcus warneri*	22050019_01
B2	*Escherichia coli*	22050019_02
B2	*Glutamicibacter arilaitensis*	22050019_03
B3	*Escherichia coli*	22050020_01
B3	*Acinetobacter parvus*	22050020_02
B4	*Enterococcus gallinarium*	22050021_01

B4	*Acinetobacter pseudolwoffii*	22050021_02
B5	*Staphylococcus pasteuri*	22050022_01
B5	*Aeromonas hydrophila*	22050022_02
B5	*Desemzia incerta*	22050022_03
B5	*Acinetobacter pseudolwoffii*	22050022_04
B6	*Bacillus licheniformis*	22050023_01
B6	*Acinetobacter lwoffii*	22050023_02
B6	*Staphylococcus equorum*	22050023_03
B6	*Aerococcus viridans*	22050023_04
B7	*Enterococcus gallinarium*	22050024
B8	*Staphylococcus equorum*	22050025
B9	*Psychrobacter pulmonis*	22050026_01
B9	*Escherichia coli*	22050026_02
B9	*Staphylococcus equorum*	22050026_03
B10	*could not be identified on maldi*	No identification
B11	*Aerococcus viridans*	22050028_01
B11	*Glutamicibacter arilaitensis*	22050028_02
B11	*Solibacillus silvestris*	22050028_03
B11	*Staphylococcus equorum*	22050028_04
B11	*Escherichia coli*	22050028_05
B12	No growth	No growth
B13	*Escherichia coli*	22050030_01
B13	*Psychrobacter pulmonis*	22050030_02
B14	*Staphylococcus equorum*	22050031_01
B14	*Desemzia incerta*	22050031_02
B15	*Psychrobacter pulmonis*	22050032_01
B15	*Desemzia incerta*	22050032_02
B16	*Staphylococcus xylosus*	22050033_01
B16	*Enterococcus gallinarium*	22050033_02
B16	*Escherichia coli*	22050033_03
B17	*Ignatzschineria ureiclastica*	22050034_01
B17	*Staphylococcus equorum*	22050034_02

7.3 Phase 1 Field based assessment – MIC data for each isolate

NR means not reported

	Bacteria	Unique ID	n_amp	n_cip	n_cta	n_ctz	n_gen	n_sme	n_tet	n_tri
Before DEXA	*Escherichia coli*	22050018_01	2	0.0075	0.03	0.12	0.25	128	1	0.25
Before DEXA	*Enterococcus gallinarium*	22050018_02	1	0.0075	NR*	NR*	2	NR*	1	NR*
Before DEXA	*Staphylococcus warnei*	22050019_01	1	0.12	1	8	0.25	32	1	0.25
Before DEXA	*Escherichia coli*	22050019_02	4	0.0075	0.03	0.06	0.25	32	1	0.25
Before DEXA	*Glutamicibacter arilaitensis*	22050019_03	1	1	1	4	0.25	8	1	0.5
Before DEXA	*Escherichia coli*	22050020_01	2	0.0075	0.03	0.12	0.25	32	1	0.25
Before DEXA	*Acinetobacter pseudolwoffii*	22050021_02	1	0.015	0.12	0.25	0.25	8	1	4
Before DEXA	*Staphylococcus pasteuri*	22050022_01	1	0.12	1	8	0.25	8	1	0.5
Before DEXA	*Acinetobacter pseudolwoffii*	22050022_04	1	0.015	0.12	0.25	0.25	8	1	2
Before DEXA	*Acinetobacter lwoffii*	22050023_02	1	0.03	0.25	0.5	0.25	8	1	8
Before DEXA	*Staphylococcus equorum*	22050023_03	1	0.12	1	8	0.25	8	1	0.25
Before DEXA	*Aerococcus viridans*	22050023_04	1	1	0.12	4	1	32	1	0.25
Before DEXA	*Enterococcus gallinarium*	22050024	1	0.25	NR*	NR*	2	NR*	1	NR*

Before DEXA	*Staphylococcus equorum*	22050025	1	0.12	2	8	0.25	32	1	0.25
Before DEXA	*Psychrobacter pulmonis*	22050026_01	1	0.0075	0.06	0.06	0.25	8	1	8
Before DEXA	*Escherichia coli*	22050026_02	2	0.0075	0.06	0.12	0.25	64	1	0.5
Before DEXA	*Staphylococcus equorum*	22050026_03	1	0.12	0.5	4	0.25	8	1	0.25
Before DEXA	*Aerococcus viridans*	22050028_01	1	1	0.25	8	0.25	8	1	0.25
Before DEXA	*Glutamicibacter arilaitensis*	22050028_02	1	1	1	8	0.25	8	1	0.5
Before DEXA	*Staphylococcus equorum*	22050028_04	1	0.12	1	8	0.25	16	1	0.5
Before DEXA	*Escherichia coli*	22050028_05	4	0.0075	0.12	0.12	0.25	32	1	0.25
Before DEXA	*Escherichia coli*	22050030_01	4	0.0075	0.06	0.06	0.25	64	1	0.25
Before DEXA	*Psychrobacter pulmonis*	22050030_02	1	0.0075	0.015	0.06	0.25	512	1	16
Before DEXA	*Staphylococcus equorum*	22050031_01	1	0.25	2	16	0.25	64	1	0.5
Before DEXA	*Psychrobacter pulmonis*	22050032_01	1	0.015	0.03	0.06	0.25	8	1	16
Before DEXA	*Staphylococcus xylosus*	22050033_01	1	0.25	1	8	0.25	32	32	0.5
Before DEXA	*Enterococcus gallinarium*	22050033_02	1	1	NR*	NR*	4	NR*	1	NR*
Before DEXA	*Escherichia coli*	22050033_03	4	0.0075	0.06	0.12	0.25	8	2	0.25
Before DEXA	*Ignatzschineria ureiclastica*	22050034_01	1	0.015	0.015	0.06	0.25	8	1	0.25

Before DEXA	*Staphylococcus equorum*	22050034_02	1	0.12	1	4	0.25	16	1	0.5
After DEXA	*Enterococcus gallinarium*	22050002	1	0.5	NR*	NR*	2	NR*	2	NR*
After DEXA	*Escherichia coli*	22050003	4	0.015	0.12	0.12	0.25	32	1	0.25
After DEXA	*Acinetobacter lwoffii*	22050004_01	1	0.015	0.5	1	0.25	8	1	8
After DEXA	*Staphylococcus equorum*	22050004_02	1	0.12	0.5	4	0.25	32	1	0.25
After DEXA	*Acinetobacter lwoffii*	22050005	1	0.015	0.12	0.25	0.25	8	1	4
After DEXA	*Acinetobacter pseudolwoffii*	22050006_02	1	0.0075	0.25	0.06	0.25	8	1	1
After DEXA	*Acinetobacter schindleri*	22050007	1	0.03	0.25	1	0.25	8	1	0.25
After DEXA	*Enterococcus gallinarium*	22050008	1	0.0075	NR*	NR*	0.25	NR*	1	NR*
After DEXA	*Psychrobacter pulmonis*	22050011_01	1	0.0075	0.015	0.06	0.25	8	1	16
After DEXA	*Enterococcus casseliflavus*	22050011_02	1	0.12	NR*	NR*	1	NR*	1	NR*
After DEXA	*Staphylococcus equorum*	22050011_03	1	0.25	1	4	0.25	32	1	0.25
After DEXA	*Psychrobacter pulmonis*	22050013_01	1	0.0075	0.015	0.06	0.25	8	1	8
After DEXA	*Acinetobacter lwoffii*	22050013_02	1	0.015	0.5	1	0.25	8	1	8
After DEXA	*Psychrobacter pulmonis*	22050014_01	1	0.015	0.12	0.06	0.25	8	1	16
After DEXA	*Aerococcus viridans*	22050014_02	1	1	0.25	8	0.25	8	1	0.25
After DEXA	*Escherichia coli*	22050014_03	1	0.0075	0.015	0.12	0.25	32	1	0.25
After DEXA	*Glutamicibacter arilaitensis*	22050014_04	1	1	0.5	4	0.25	8	1	1
After DEXA	*Ignatzschineria ureiclastica*	22050015_01	1	0.015	0.015	0.06	0.25	8	1	0.25
After DEXA	*Glutamicibacter protophormiae*	22050015_02	1	1	0.25	4	0.25	8	1	0.25
After DEXA	*Acinetobacter lwoffii*	22050016_02	1	0.015	0.25	0.5	0.25	8	1	8
After DEXA	*Escherichia coli*	22050017_01	1	0.0075	0.12	0.12	0.25	32	1	0.25
After DEXA	*Ignatzschineria indica*	22050017_02	1	0.0075	0.015	0.06	0.5	8	1	2

7.4 Phase 3 Field based assessment – MIC data for each isolate

	n_amp	n_cip	n_cta	n_ctz	n_gen	n_sme	n_tet	n_tri
kleb_C.bmp	32	0.03	0.03	0.25	0.25	512	1	1
kleb_T1.bmp	32	0.03	0.03	0.12	0.25	512	1	1
kleb_T2.bmp	32	0.03	0.03	0.12	0.25	512	1	1
kleb_T3.bmp	32	0.015	0.03	0.25	0.25	512	1	1
kleb_T4.bmp	32	0.015	0.03	0.12	0.25	512	1	0.5
kleb_T5.bmp	32	0.03	0.03	0.12	0.25	512	1	1
kleb_T6.bmp	32	0.03	0.03	0.12	0.25	512	1	1
kleb_T7.bmp	32	0.015	0.03	0.12	0.25	512	1	1
kleb_T8.bmp	32	0.03	0.03	0.25	0.25	512	1	1
atccEF_C.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T1.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T2.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T3.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T4.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T5.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T6.bmp	1	0.5	4	16	4	512	32	0.25
atccEF_T7.bmp	1	0.5	4	16	4	512	16	0.25
atccEF_T8.bmp	1	0.5	4	16	4	512	32	0.25
atccS_C.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T1.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T2.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T3.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T4.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T5.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T6.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T7.bmp	1	0.5	2	16	0.25	512	1	1
atccS_T8.bmp	1	0.5	2	16	0.25	512	1	1
gui_C.bmp	32	0.12	4	2	0.25	8	1	16
gui_T1.bmp	16	0.12	4	2	0.25	8	1	16
gui_T2.bmp	16	0.12	4	2	0.25	8	1	16
gui_T3.bmp	16	0.12	4	2	0.25	8	1	16
gui_T4.bmp	16	0.12	4	2	0.25	8	1	16
gui_T5.bmp	16	0.12	4	2	0.25	8	1	16
gui_T6.bmp	16	0.12	4	2	0.25	8	1	16
gui_T7.bmp	16	0.12	4	2	0.25	8	1	16
gui_T8.bmp	16	0.12	4	2	0.25	8	1	16
pumi_C.bmp	1	0.25	4	16	0.25	8	1	0.25

pumi_T1.bmp	1	0.25	4	16	0.25	8	1	0.25
pumi_T2.bmp	1	0.25	4	16	0.25	8	1	0.25
pumi_T3.bmp	1	0.5	4	16	0.25	8	1	0.25
pumi_T4.bmp	1	0.25	4	16	0.25	8	1	0.25
pumi_T5.bmp	1	0.25	4	16	0.25	8	1	0.25
pumi_T6.bmp	1	0.12	4	16	0.25	8	1	0.25
pumi_T7.bmp	1	0.25	4	16	0.25	8	1	0.25
pumi_T8.bmp	1	0.25	4	16	0.25	16	1	0.25
sap_C.bmp	1	0.5	4	16	0.25	512	1	1
sap_T1.bmp	1	0.5	4	16	0.25	512	1	1
sap_T2.bmp	1	0.5	4	16	0.25	512	1	1
sap_T3.bmp	1	0.5	4	16	0.25	512	1	1
sap_T4.bmp	1	0.5	4	16	0.25	512	1	1
sap_T5.bmp	1	0.5	4	16	0.25	512	1	1
sap_T6.bmp	1	0.25	4	16	0.25	512	1	1
sap_T7.bmp	1	0.5	4	16	0.25	512	1	1
sap_T8.bmp	1	0.5	4	16	0.25	512	1	1
div_C.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T1.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T2.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T3.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T4.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T5.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T6.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T7.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
div_T8.bmp	1	0.0075	0.015	0.06	0.25	8	1	0.25
equ_C.bmp	1	0.25	4	16	0.25	512	1	2
equ_T1.bmp	1	0.25	4	16	0.25	512	1	2
equ_T2.bmp	1	0.25	4	16	0.25	512	1	2
equ_T3.bmp	1	0.25	4	16	0.25	512	1	2
equ_T4.bmp	1	0.25	4	16	0.25	512	1	2
equ_T5.bmp	1	0.25	4	16	0.25	512	1	4
equ_T6.bmp	1	0.25	4	16	0.25	512	1	2
equ_T7.bmp	1	0.25	4	16	0.25	512	1	2
equ_T8.bmp	1	0.25	4	16	0.25	512	1	2
hirae_C.bmp	1	2	4	16	4	512	1	0.25
hirae_T1.bmp	1	2	4	16	4	512	1	0.25
hirae_T2.bmp	1	2	4	16	4	512	1	0.25
hirae_T3.bmp	1	2	4	16	4	512	1	0.25
hirae_T4.bmp	1	2	4	16	4	512	1	0.25
hirae_T5.bmp	1	2	4	16	4	512	1	0.25

hirae_T6.bmp	1	2	4	16	4	512	1	0.25
hirae_T7.bmp	1	2	4	16	4	512	1	0.25
hirae_T8.bmp	1	2	4	16	4	512	1	0.25
1302E_C.bmp	2	0.0075	0.06	0.06	0.5	16	1	0.25
1302E_T1.bmp	2	0.0075	0.03	0.12	0.5	16	1	0.25
1302E_T2.bmp	2	0.0075	0.03	0.12	0.5	16	1	0.25
1302E_T3.bmp	2	0.0075	0.03	0.12	1	32	1	0.25
1302E_T4.bmp	2	0.0075	0.03	0.12	0.5	16	1	0.25
1302E_T5.bmp	2	0.0075	0.06	0.12	1	32	1	0.25
1302E_T6.bmp	2	0.0075	0.03	0.12	0.25	32	1	0.25
1302E_T7.bmp	2	0.0075	0.06	0.12	0.5	32	1	0.25
1302E_T8.bmp	2	0.0075	0.03	0.12	0.25	32	1	0.25
402E_C.bmp	2	0.0075	0.06	0.25	0.25	512	2	0.25
402E_T1.bmp	2	0.0075	0.06	0.12	0.5	512	2	0.25
402E_T2.bmp	2	0.0075	0.06	0.12	0.5	512	2	0.5
402E_T3.bmp	2	0.0075	0.06	0.12	0.25	512	2	0.5
402E_T4.bmp	2	0.015	0.06	0.12	1	512	2	0.25
402E_T5.bmp	2	0.0075	0.06	0.12	1	512	2	0.5
402E_T6.bmp	2	0.0075	0.06	0.25	1	512	2	0.5
402E_T7.bmp	2	0.0075	0.06	0.25	0.5	512	2	0.5
402E_T8.bmp	2	0.0075	0.06	0.12	0.25	512	2	0.25
atccE_C.bmp	4	0.0075	0.12	0.12	1	512	2	0.5
atccE_T1.bmp	4	0.0075	0.12	0.25	1	512	1	0.5
atccE_T2.bmp	8	0.0075	0.12	0.25	1	512	1	0.5
atccE_T3.bmp	4	0.0075	0.12	0.12	1	512	1	0.5
atccE_T4.bmp	4	0.0075	0.12	0.12	0.5	512	1	0.5
atccE_T5.bmp	4	0.0075	0.12	0.12	0.5	512	1	0.5
atccE_T6.bmp	8	0.0075	0.12	0.25	0.5	512	1	0.5
atccE_T7.bmp	4	0.0075	0.06	0.12	0.5	512	1	0.5
atccE_T8.bmp	4	0.0075	0.12	0.12	0.25	512	1	0.5
bau_C.bmp	16	0.12	2	1	0.25	8	2	8
bau_T1.bmp	8	0.12	2	1	0.25	8	2	8
bau_T2.bmp	16	0.12	4	1	0.5	8	2	8
bau_T3.bmp	8	0.12	2	0.5	0.25	8	2	8
bau_T4.bmp	8	0.12	2	1	0.25	8	2	8
bau_T5.bmp	8	0.12	2	1	0.5	8	2	8
bau_T6.bmp	8	0.12	2	1	0.25	8	2	8
bau_T7.bmp	8	0.12	2	1	0.25	8	1	8
bau_T8.bmp	8	0.12	2	1	0.25	8	2	8