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In this issue:

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FROM THE EDITOR

In this edition of the Breakpoints Newsletter, we showcase recent outstanding infectious diseases and antimicrobial clinical trial research led by Australian researchers, including articles from SNAP, BLING and ASAP / CALIPSO research teams.

We congratulate Cindy Lau on the completion of her PhD detailing linezolid and cefepime toxicity and the role of TDM.

ASA recently hosted the 19th International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) in Perth. Geoffrey Coombs and Shakeel Mowlaboccus provide a summary and we include the original Australian research posters that were presented at the conference.

The Australian Committee on Antimicrobial Susceptibility Testing (AUSCAST) present their first endorsed Guidance Document, detailing anaerobic disk diffusion and how to implement this in your clinical laboratory.

Finally, we welcome-back our microbiology photo quiz article. We hope you enjoy the newsletter.

ASA's Annual Scientific Meeting, Antimicrobials 2025, is being held in Melbourne from 20 - 22nd February. Plenary speakers including Christian Giske, Erin McCreary and Rachel Thomson. The Howard Florey oration will feature Karin Thursky.

During Antimicrobials 2025, six oral proffered paper sessions have been scheduled. ASA has made funds available for travel awards to assist members wanting to attend the ASM. Abstract submission deadline is Friday 13th December. ASA members who wish to apply for this award are invited to submit their application to the ASA Secretary at info@asainc.net.au. The application should include a copy of the abstract and for abstracts with more than one author, a letter stating the relative contribution of the applicant towards the research.

The 2025 Australian Society for Antimicrobials (ASA) Annual Keryn Christiansen Research Grant of up to $25,000 to support original research is now available. Apply online at the ASA website.

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front cover: https://www.pexels.com/photo/blood-samples-in-test-tubes-6622515/

PHOTO QUIZ

A 56-year-old woman presented to ED with symptoms consistent with acute cystitis that has failed to improve with a 7-day course of ciprofloxacin administered by her GP. A previous uncomplicated UTI one year previously had afforded an ESBL producing *E. coli* which was susceptible to ciprofloxacin via Phoenix and she had clinically improved with this therapy. For her most recent presentation urine was sent for microscopy and culture which identified >100 x 10^6 /L leukocytes and growth of an *Escherichia coli* overnight. Given her previous results and being systemically well with no evidence of pyelonephritis, she was discharged on IV ertapenem via hospital in the home with a peripheral IVC for a presumed ESBL producing *E. coli* which had failed to improve with oral ciprofloxacin.

Phoenix susceptibilities on the NMIC422 panel confirmed an ESBL phenotype with cefepime resistance (MIC 16 mg/L) as well as susceptibility to ertapenem (MIC ≤0.25 mg/L). Oral options including ciprofloxacin (MIC >4 mg/L), nitrofurantoin (MIC >64 mg/L) and trimethoprim (>8 mg/L) were resistant, whilst the fosfomycin MIC was reported as ≤16 mg/L. The isolate was submitted to agar dilution to confirm fosfomycin result with the results noted below in Figure 1.

How would you interpret the fosfomycin susceptibility testing?

What other methods for fosfomycin AST are validated?

How could the testing be interpreted were the organism a Klebsiella pneumoniae rather than E. coli?

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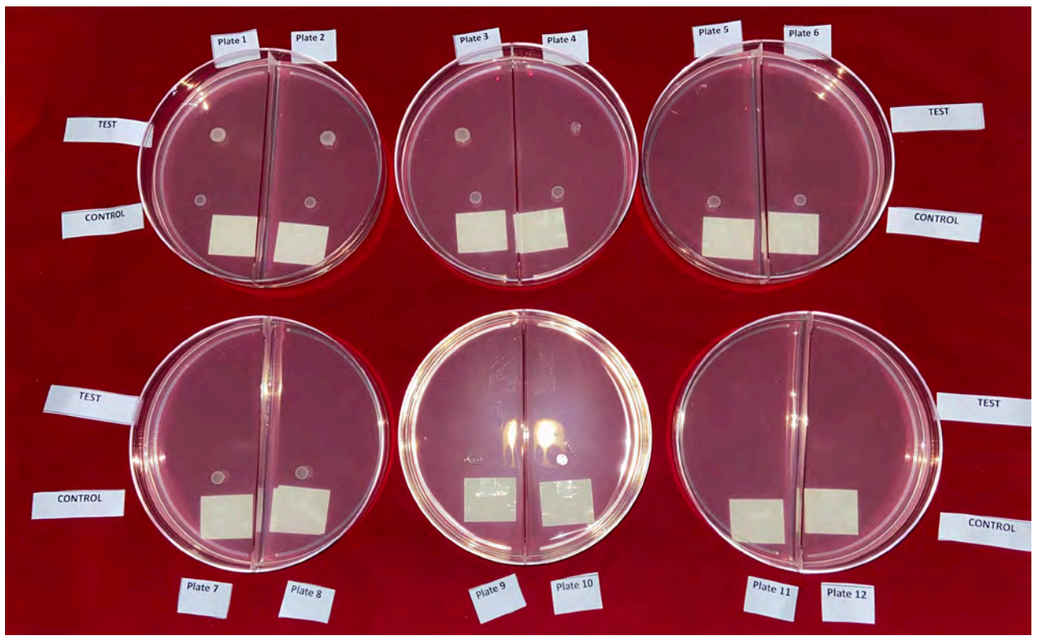

Plate number	1	2	3	4	5	6	7	8	9	10	11	12
Fosfomycin concentration (µg/mL)	0	0.25	0.5	1.0	2.0	4.0	8.0	16	32	64	128	256

IN THE SPOTLIGHT

Cefepime & Linezolid Toxicity & Therapeutic Drug Monitoring

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Introduction

Antimicrobial resistance is considered one of the most serious global health threats, with infections caused by resistant pathogens becoming more difficult to treat effectively [1]. Sufficient dosing of last-line antimicrobial agents is essential; however these agents are often associated with more serious adverse effects. Dosing must balance maintaining effective plasma concentrations while minimising toxicity risk. Strategies to optimise this balance are required. Cefepime and linezolid are two essential antimicrobial agents where serious toxicities may be associated with drug exposure, and as such therapeutic drug monitoring (TDM) may be beneficial to optimise efficacy while avoiding toxicity. My PhD aimed to explore the relationship between cefepime and linezolid exposures and toxicity in a clinical setting, and the potential impact of TDM on minimising toxicity.

Research Studies

Cefepime

We performed a retrospective review of adult patients administered cefepime in a tertiary referral hospital between October 2017 and May 2018, aiming to define a cefepime toxicity threshold, and identify the incidence of and patient risk factors associated with the development of cefepime-induced neurotoxicity. Neurotoxicity was identified by the documentation of neurological symptoms of any grade in the patient's progress notes according to the National Cancer Institute's (NCI) [2] classification. Neurotoxicity attributed to cefepime therapy was then defined based on the conditions specified by the World Health Organisation, Uppsala Monitoring Centre (WHO-UMC) causality assessment criteria [3], and reviewed independently by two investigators.

Of the 259 courses administered, there was an overall incidence of cefepime-induced neurotoxicity of 6% (16/259 courses). The most common clinical feature which defined CIN in our population was confusion. The median time from commencement of cefepime therapy to the onset of neurotoxicity was 84 hours (IQR 58 – 131). A multivariable logistic regression identified only the cefepime trough concentration was significantly associated with the occurrence of cefepime-induced neurotoxicity (Table 1)[4].

	Multivariable	
	Adjusted Odds Ratio [95% CI]	p-value
Age (years)	N/A	N/A
Weight (kg)	N/A	N/A
Serum creatinine at start of therapy (μmol/L)	1.00 [0.99, 1.02]	0.42
Duration of cefepime therapy (days)	0.85 [0.58, 1.26]	0.42
Total cefepime administered in course (g)	N/A	N/A
Average daily dose of cefepime (g)	N/A	N/A
Cefepime trough plasma concentration (mg/L)	1.06 [1.02, 1.10]	< 0.01

Table 1. Multivariable logistic regression of patient covariates predicting likelihood of experiencing cefepime-induced neurotoxicity

SPECIFIC AIMS

1 Identify the frequency and factors associated with the development of cefepime-induced neurotoxicity and linezolid toxicity

2 Refine the cefepime toxicity threshold

3 Determine the impact of linezolid therapeutic drug monitoring on linezolid toxicity

As only 64 courses had an accurate cefepime trough (C_{min}) concentration measured (24.7%), a cefepime population pharmacokinetic model was validated using our data[5], and then utilised to determine cefepime C_{min}, C_{max} and AUC_{24} for the 102 courses where any cefepime concentration was measured. Receiver-Operating Characteristic (ROC) curve analyses were performed on each pharmacokinetic parameter to determine the cefepime parameter most predictive of cefepime-induced neurotoxicity. A cefepime C_{min} of 49mg/L was identified as the most appropriate toxicity threshold based on the predictive ability (Area Under the ROC [AUROC] 0.88, 95%CI 0.76-0.99, p < 0.001), and clinical feasibility (Figure 1)[6].

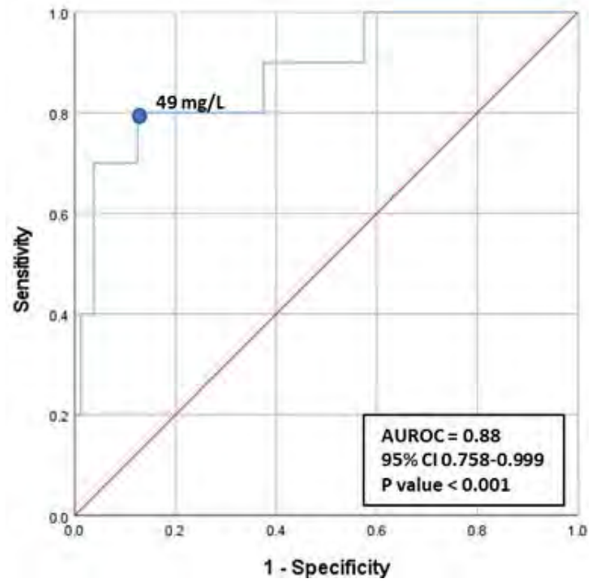

Figure 1 Receiver-operating Characteristic (ROC) curve analysis to predict neurotoxicity from highest cefepime C_{min}

Given that baseline serum creatinine was significantly associated with cefepime-induced neurotoxicity in the univariable analysis (p = 0.02) despite appropriate renal dose adjustment in this cohort, we were interested to review whether the Australian renal dosage adjustment guidelines [7] were appropriate. Monte Carlo simulations using the validated population pharmacokinetic model were performed to evaluate the ability of Australian cefepime dosing recommendations to achieve the established efficacy target of C_{min} > 32 mg/L (based on the pharmacokinetic/pharmacodynamics target of 4 times the breakpoint minimum inhibitory concentration of *Pseudomonas aeruginosa*) [8], whilst maintaining exposure below the identified toxicity threshold of 49 mg/L. Predicted concentration-time profiles for a representative simulated patient population of 12,000 individuals administered cefepime according to renal adjustment guidelines were constructed. Overall, only 29% of simulated patients were expected to achieve the target range, with simulated patients with impaired renal function more likely to exhibit sub-therapeutic cefepime concentrations (Figure 2). This unexplained result may be due to excessively conservative renal dose reductions, and requires further investigation.

We next conducted a systematic review investigating studies that had defined a cefepime toxicity threshold from data base inception until December 2019. There were 9 eligible studies identified from 5 different countries. Individual patient data was obtained from 4 of the 9 studies (407 patients), of which 89 experienced cefepime-induced neurotoxicity (22%). Individual patient data meta-analysis identified an optimal cutoff to be 34.5mg/L (sensitivity 0.78 [95%CI 0.38-0.95], specificity 0.90 [95%CI 0.62-0.98], AUROC 0.89 [95%CI 0.75-0.96]). However, this is not considered to be a clinically viable threshold given that the efficacy target ≤32 mg/L [8], and an updated review is required to refine this threshold further.

	Multivariable logistic regression	
	Adjusted Odds Ratio [95% CI]	p-value
Serum creatinine at baseline (µmol/L)	1.00 [1.00-1.00]	0.20
Platelets at baseline (x 10^9/L)	1.00 [1.00-1.00]	0.008
Duration of linezolid > 28 days	1.42 [0.85-2.36]	0.18
Any linezolid TDM performed	3.45 [1.88-6.33]	<0.001
Appropriate dose adjustment with first TDM	0.45 [0.21-0.96]	0.038

Table 2: Multivariable logistic regression of patient covariates predictive of linezolid toxicity

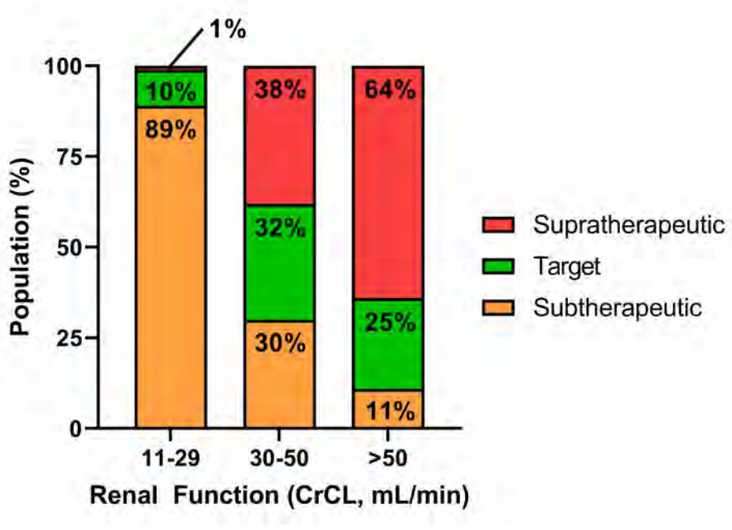

Figure 2: The predicted proportion of simulated individuals within the cefepime therapeutic range based on cefepime administration according to current dosing guidelines.

Linezolid

Linezolid is another example of an important antimicrobial agent with a clear relationship between supratherapeutic linezolid plasma concentrations and toxicity, with a haematological toxicity threshold of 7 mg/L clearly defined in previous publications [9]. However, there are limited clinical studies investigating the potential impact of performing linezolid TDM on patient clinical outcome. We performed a retrospective multi-centre review of 1050 patients administered linezolid between January 2017 and December 2019 in 11 hospitals [10]. Of the 622 patients included, 105 (16.9%) were assessed to have experienced treatment-limiting linezolid toxicity, requiring premature cessation of intended treatment. These patients displayed a higher baseline creatinine, lower platelet count, and received a longer linezolid course than patients who did not experience toxicity. The most common toxicity experienced was haematological (thrombocytopaenia and anaemia).

Linezolid TDM was performed in 144 / 622 patients (23%), of whom 120 had sampling performed in the first linezolid dosage regimen. The median first concentration measured was 8.25 mg/L (IQR 2.75-14.78), and 86/120 patients had appropriate dose adjustments (72%). A multivariable model demonstrated that TDM-guided appropriate dose adjustment significantly reduced the odds of a patient experiencing linezolid toxicity (aOR=0.45, 95%CI 0.21-0.95, p = 0.038) (Table 2).

Key findings and recommendations

- There is a high frequency of serious cefepime and linezolid toxicities, resulting in unplanned treatment deviation or cessation
- There is a clear association between cefepime and linezolid plasma concentrations and toxicity
- Cefepime trough plasma concentration was the only statistically significant patient variable contributing to cefepime-induced neurotoxicity
- Cefepime renal dose adjustment based on current guidelines may not achieve therapeutic concentrations
- A cefepime toxicity threshold of 49 mg/L should be considered in clinical settings to diagnose and potentially prevent the occurrence of cefepime-induced neurotoxicity
- TDM-guided dose adjustment of linezolid reduced the likelihood of the development of treatment-limiting linezolid toxicity

Closing remarks

- Performing cefepime and linezolid therapeutic drug monitoring should reduce the risk of developing toxicity whilst maintaining efficacy
- Antimicrobial stewardship pharmacists are well placed to utilise cefepime and linezolid therapeutic drug monitoring to optimise therapy

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AUSTRALIAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
Australian Society for Antimicrobials

Disk diffusion testing for anaerobes

Brooke Webb & Teresa Abajo
on behalf of the Australian Committee on Antimicrobial Susceptibility Testing (AUSCAST)

Background

Historically, antimicrobial susceptibility testing (AST) of anaerobes has not been required because infection with these bacteria was often able to be treated adequately with empiric therapy and/or surgical intervention. Increasing rates of treatment failures have been linked to emerging resistance to many of the agents used in empirical therapy [1–3]. In the near future, AST of anaerobes will be necessary to guide therapy.

It is suggested that anaerobic AST should be considered in the following scenarios [4]:

- A positive blood culture where an anaerobe has been isolated
- An anaerobe is isolated from a normally sterile site
- An infection involving anaerobes that is not responding to empirical treatment.

Periodic surveillance of commonly seen anaerobes could also be considered by individual laboratories. This would enable elucidation of the local antibiogram, and such data could be fed into Australian Passive AMR Surveillance (APAS). This data enables monitoring of geographical and organism-related trends.

EUCAST has developed clinical breakpoints for selected rapidly growing anaerobic bacteria for disk diffusion [5]. This is allowing more laboratories to introduce anaerobic AST using a familiar and cost-effective method, without the need to perform reference standard agar dilution, broth microdilution or to referring isolates to reference laboratories.

Breakpoints are available for a select list of commonly encountered organisms:

- *Bacteroides* spp.
- *Prevotella* spp.
- *Fusobacterium necrophorum*
- *Clostridium perfringens*
- *Cutibacterium acnes*

The range of antibiotics for which breakpoints are provides options from several antibiotic classes relevant to Australian formularies. Zone diameter breakpoints for *Clostridioides difficile* are in development.

Introduction of anaerobic AST to a laboratory

Things to consider when contemplating the introduction of disk diffusion testing of anaerobes by the EUCAST method include:

1. Creation of the anaerobic atmosphere. Most laboratories would already have jars, boxes or chambers if they are culturing for anaerobes.
2. Procurement of the organisms required for the quality control.
3. Procurement or manufacture of the required medium. For disk diffusion of anaerobes, the EUCAST method requires Fastidious Anaerobe Agar with defibrinated horse blood (FAA-HB).
4. Procurement of the required antimicrobial disks in the concentrations stipulated in current EUCAST breakpoint tables.
5. Perform verification by testing the QC organisms against each of the antimicrobials that the laboratory is proposing to introduce.
6. Appointing designated staff to perform testing, review results, write up a report and deliver training. This should include experienced AST scientists as well as clinicians.
7. Identification and implementation of changes to reporting panels in the local laboratory information system (LIS).

There is no requirement for laboratories to offer all antimicrobials for all the organisms listed. A reasonable place to start for most laboratories to commence AST for anaerobes is metronidazole for *Bacteroides* spp. These

species are among the most common anaerobes isolated from human infections [6], and while currently resistance rates remain low [7], there are increasing reports of the emergence of resistance that will make AST a necessity in the near future.

In-lab verification

The introduction of a new method to a laboratory involves the verification of the method in the hands of the user. If the test can be shown to work reliably in the hands of the user, it can be considered verified. For most laboratories where disk diffusion is already performed, anaerobic AST is not strictly speaking, a new method. Therefore, a validation process should include, for example, testing each QC isolate against each of the selected antimicrobial disks in triplicate (i.e. creating a fresh 1.0McF suspension each time), on 5 separate occasions (days), preferably including different operators (Fig. 1).

Quality Control of anaerobic environment

In addition to routine quality control of antibiotic disks, the quality of the anaerobic environment must also be monitored with every anaerobic AST.

Colour change with chemical indicators is often used as a control of the anaerobic environment; however, they can remain negative although low levels of oxygen are present. Also, they can only display the actual atmosphere and cannot provide information on changes in oxygen concentration over time. The growth of strict anaerobic bacteria can be used as a biological indicator, but failure of the

Verification of anaerobic AST

Set up date/initial: 12/9/24 TA 1330 hrs Read date/initial: 13/9/24 TA 0930 hrs

(Perform on 5 different days, separate inoculum for each of 3 tests)

***Bacteroides fragilis* ATCC 25285** Media batch/expiry 4472/73 23/9/24

Antibiotic Disk label	Disk batch/exp	Zone mm Test 1	Test 2	Test 3	Expected diameter (range mm)	Pass/fail
AMC3	6142382 31/7/27	25	26	25	23-29	pass
DA2	6164433 29/8/27	26	28	27	23-29	pass
MEM10	6172436 11/9/25	36	35	35	32-39	pass
MTZ5	6150775 31/8/27	32	34	34	29-36	pass
TZP36	6154454 31/8/27	31	33	34	29-35	pass

***Clostridium perfringens* ATCC 13124** Media batch/expiry 4472/73 23/9/24

Antibiotic Disk label	Disk batch/exp	Zone mm Test 1	Test 2	Test 3	Expected diameter (range mm)	Pass/fail
AMP2	6160726 20/8/27	30	33	32	29-35	pass
AMC3	6142382 31/7/27	33	31	32	28-34	pass
P1	6154424 31/8/27	24	26	27	23-29	pass
CTX5	6150786 31/8/25	28	29	31	27-33	pass
CRO30	6110037 1/5/27	35	32	34	31-37	pass
DA2	6164433 29/8/27	21	22	24	20-26	pass
LZD10	6154456 15/8/26	25	26	25	22-28	pass
MEM10	6172436 11/9/25	35	37	37	34-40	pass
MTZ5	6150775 31/8/27	24	21	23	20-26	pass
TZP36	6154454 31/8/27	34	32	34	30-36	pass
VAN5	6162988 31/8/27	17	16	16	14-20	pass

Figure 1 Example of the documentation of the verification. Consider performing with a second operator

control organism to grow can be due to the presence of oxygen or that the strain was already nonviable at the time of inoculation.

To indicate that the environment is of sufficient quality, a suspension of an aerotolerant *Clostridium perfringens* strain DSM 25589 (CCUG 75076 and NCTC 14679) is inoculated onto a plate with a 5 µg metronidazole disk. Metronidazole is dependent on partial reduction in an anaerobic environment to be active. A strict anaerobic environment is necessary to obtain confluent growth with the *C. perfringens* strain and a metronidazole zone diameter above the cut-off value of 25mm. Even a very low concentration of oxygen can cause the expected zone diameter of a metronidazole disk to decrease markedly, making this method a sensitive indicator of adequate anaerobicity.

Performance of disk diffusion testing of anaerobes

EUCAST have a published method for the AST of anaerobes using disk diffusion (www.eucast.org) [5].

Some points of difference to note about testing anaerobes using the EUCAST method are listed below:

- The density of the inoculum suspension needs to be McFarland 1.0
- Any excess moisture on the plates (condensation) will result in fuzzy or irregular zones of inhibition around the antibiotic disks. It is recommended plates be dried well and be at room temperature before inoculation. Instructions for this are included in the method [5].
- For *Bacteroides* spp. and *C. perfringens*, remove excess fluid by turning the swab against the inside of the tube to avoid over-inoculation. Do not remove excess fluid for *F. necrophorum*, *Prevotella* spp. and *C. acnes*.
- Confluent lawn cultures are particularly important for *C. acnes* and some *Prevotella* spp., that grow small colonies on FAA-HB. Gaps between streak lines will result in irregular zones of inhibition that are difficult to read.
- The number of disks on each plate must be limited. Three disks maximum per 90mm plate, and in the case of *C. acnes*, 1-2 disks per plate provides better clarity. The zones for anaerobes and these antibiotics can be large, and overcrowding of plates will result in overlapping zones that are difficult to read.
- The method has been validated for 18 hours +/- 2 hours, and should not be read before 16 hrs, or after 20 hrs.

Reading of anaerobe disk diffusion plates

Instructions for reading zones of inhibition are included in the method [5]. In addition, a reading guide [8] is available that contains photographs to help reading zones of inhibition (Figures 2-4).

It is recommended that a consensus reading of zones be undertaken by at least two operators for accurate measurement, recording and interpretation of zone diameters, especially in the context of fine, hard to detect growth and lack of experience with new methodology.

Automated zone readers (e.g. BIOMIC) can lack the resolution required to accurately read very fine growth such as that of *C. acnes*. Such systems are still useful for the recording of manually read zone diameters with automated categorical interpretation and transfer of results to the LIS.

Should aberrant results arise during the verification procedure that can't be explained by procedural errors, it would be useful for the laboratory to have access to the reference method. The EUCAST reference method for anaerobes is agar dilution using the same medium as disk diffusion.

Conclusion

EUCAST methodology for AST of anaerobes using disk diffusion is accessible, cost effective and easy to perform. We recommend laboratories consider implementing anaerobic AST to have a positive impact on patient care.

For laboratories new to EUCAST, planning will be required for a broader verification utilising different organisms and methodologies. A guide to assist laboratories to transition to EUCAST can be found on the EUCAST website.

Local advice can be sought from AUSCAST at auscastasa@gmail.com

Figure 2 *Bacteroides* spp. and meropenem double zone. In the case of double zones the inner zone edge should be read

Figure 3 Tiny colonies of *C. perfringens* within the clindamycin zone. The zone of inhibition must be examined closely for any colonies which must be considered when measuring the zone

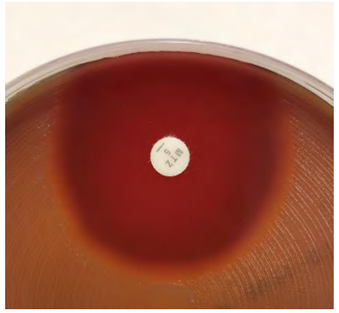

Figure 4 Haemolysis of *F. necrophorum* at edge of metronidazole zone of inhibition. Ignore haemolysis and swarming

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8 Reading guide EUCAST disk diffusion for selected rapidly growing anaerobic bacteria on Fastidious Anaerobe Agar with 5% horse blood (FAA-HB) Version 2.0. 2023.

QUIZ ANSWER

How would you interpret the fosfomycin susceptibility testing?

The fosfomycin MIC is 2mg/L via agar dilution, representing a susceptible isolate.

What other methods for fosfomycin AST are validated?

Disk diffusion is also a validated method for AST for fosfomycin AST for *E. coli* using a 200 µg/L fosfomycin disk containing 50µg of glucose-6-phosphate, although there are differences in interpretation between EUCAST and CLSI.

How could the testing be interpreted were the organism a Klebsiella pneumoniae rather than E. coli?

There are no susceptibility interpretative criteria for species other than *E. coli* under either EUCAST or CLSI.

The fosfomycin MIC is 2 mg/L via agar dilution, representing a susceptible isolate. CLSI breakpoints for fosfomycin susceptibility are defined as ≤64 mg/L [1], whilst EUCAST has recently updated for IV fosfomycin from ≤32 mg/L to ≤8 mg/L to match PO fosfomycin [2]. Importantly, both EUCAST and CLSI recommend agar dilution as the MIC reference method rather than broth microdilution given unacceptably high rates of major error (ME) and very major error (VME). One study by van den Bijllaardt et al. from the Netherlands [3] noted VME rates of 18.8% for Vitek2 and 12.5% for Phoenix, with lower ME rates of 0.3% and 0.0% respectively when compared with agar dilution under EUCAST v8.0 criteria from 2018.

Disk diffusion is also a validated method for AST for fosfomycin AST for E. coli using a 200 µg/L Fosfomycin disk containing 50µg of glucose-6-phosphate, although there are differences in interpretation between EUCAST and CLSI. EUCAST advises not including isolated colonies occurring within the zone of inhibition, which is set at 24mm for susceptible [2], whilst CLSI advises to include all colonies with a smaller zone of 16mm [1]. Although disk diffusion is a validated method there remains a potentially high rate of error, van den Bijllaardt et al. noting VME rates of 12.9%, and ME rates of 1.1% [3].

There are no susceptibility interpretative criteria for species other than E. coli under either EUCAST or CLSI. The majority of *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Enterobacter spp.* have intrinsic FosA enzymes which act to cleave and inactivate fosfomycin, resulting in higher ECOFFs when compared to *E. coli* and a failure to achieve PK/PD targets [4]. Neither EUCAST nor CLSI have set breakpoints for Enterobacterales other than *E. coli* [1,2]. If breakpoints are extrapolated to other Enterobacterales then it is noted that there is poor categorical agreement between EUCAST and CLSI, principally due to a high presence of isolated inner colonies [5].

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CLINICAL RESEARCH

*S*taphylococcus aureus *N*etwork *A*daptive *P*latform trial

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Approaches to the management of *Staphylococcus aureus* bacteraemia (SAB) vary widely, and basic questions remain unanswered. For example, which is the best upfront antibiotic for methicillin susceptible *S. aureus* (MSSA) bacteraemia?

Analysis of SAB datasets collated by the Australian Group on Antimicrobial Resistance (AGAR) over many years have provided invaluable descriptions of the burden of SAB disease, and the mix of resistance profiles and of *S. aureus* clones.[1] The mortality rate for each episode continues to be 15% in Australia. Retrospective observational studies using the Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP) datasets have revealed associations between mortality and treatment with cefazolin versus flucloxacillin for MSSA[2] and penicillin versus flucloxacillin for penicillin-susceptible *S. aureus* (PSSA).[3] The largest reported paediatric collection of SAB, also using ASSOP data, demonstrated high incidence in First Nations populations and found that vancomycin use compared with anti-staphylococcal beta-lactams for MSSA was associated with increased mortality.[4] As valuable as these studies have been, their observational nature necessarily limit any conclusions about comparative treatment effects of different agents or management strategies. Ultimately, randomised clinical trials are required to provide robust evidence to inform individual patient management and guidelines.

The *S. aureus* Network Adaptive Platform (SNAP) trial is designed to fill this gap by answering multiple questions within a single platform. There is a core protocol that defines inclusion and exclusion criteria and the primary outcome of 90-day mortality.[5] Inclusion criteria are simple: the patient has grown *S. aureus* in blood cultures within the past 72 hours. Exclusion criteria are minimal to allow broad enrolment. Domain specific appendices then define each specific question – in essence, these are separate trial protocols nested within the core.[6,7] At present there are three

Silo	Antibiotic Backbone Domain#	Adjunctive Treatment Domain	Early Oral Switch Domain
PSSA	(Flu)cloxacillin* Penicillin	Clindamycin vs No clindamycin*	Continued IV* versus early oral switch At 7 days At 14 days
MSSA	(Flu)cloxacillin* Cefazolin		
MRSA	Vancomycin* vs Vancomycin plus cefazolin		

Figure 1. *Staphylococcus aureus* Network Adaptive Platform (SNAP) trial design with domains and silos. The asterixis indicate the 'control' arm in each cell. PSSA: penicillin-susceptible *S. aureus*, MSSA: methicillin-susceptible *S. aureus*, MRSA: methicillin-resistant *S. aureus*. # The backbone domain for the PSSA and MSSA silos has been closed.

"The S. aureus Network Adaptive Platform (SNAP) trial is designed to address the lacking robust RCTs to inform individual patient management and guidelines. SNAP trial addresses this large research gap by answering multiple questions within a single platform."

domains (backbone antibiotic, adjunctive antibiotic, and early oral switch) for the three silos (PSSA, MSSA, MRSA) (Figure 1), although the backbone antibiotic domain for the PSSA and MSSA silos has recently been closed on the recommendation of the trial Data and Safety Monitoring Committee, and currently only participants with MRSA bacteraemia are being recruited to the backbone domain. The trial uses a Bayesian adaptive statistical framework with pre-specified rules for when any one of these domains concludes.[8] Over time, domains may be closed when a conclusion for effectiveness (based on 90-day mortality) or safety is reached, and new domains may be opened. Key innovations of the trial include a consumer directed simplified consent process (see https://www.snaptrial.com.au/),[9] and harmonised inclusion of children and pregnant women.[10]

SNAP began in 2022, initially with NHMRC funding support, and is now recruiting at 117 hospital sites in 8 countries, with >3,000 participants enrolled (Figure 2). The scale of the trial allows for rapid enrolment of participants and provides statistical power to test for reasonably small comparative treatment effects. The study design also provides early opportunities to discover either benefit or harm, through iterative scheduled analyses after every 500 participants. Here we further describe two of the active trial domains – adjunctive antibiotic and early oral switch.

SNAP Adjunctive Antibiotic Domain

Human studies involving clindamycin for exotoxin inhibition in invasive *S. aureus* infections have shown mixed results, often limited by low-quality evidence arising from case reports,[11] case series[12], or small clinical trials.[13] A recent open-label, pilot, randomized controlled trial (RCT) evaluated the efficacy of standard therapy alone versus standard therapy plus adjunctive clindamycin in adults and children with severe *S. aureus* infections. Although no difference was detected in the primary outcome of systemic inflammatory response syndrome–free days by day 14, the 90-day mortality rate was 0% (0/17) in the adjunctive clindamycin group versus 24% (4/17) in the standard therapy group.[13] While the pilot RCT was underpowered to determine the effectiveness of clindamycin, it demonstrated feasibility and provides the rationale for a larger and more robust trial.

"SNAP is now the largest trial of SAB treatment ever conducted and recruitment remains robust."

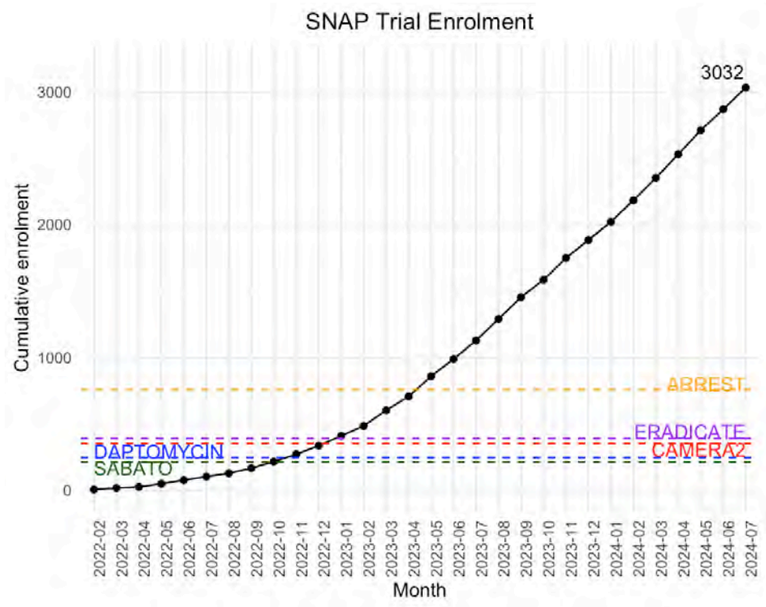

Figure 2. *Staphylococcus aureus* Network Adaptive Platform (SNAP) trial cumulative enrolment to 31 July 2024.

The adjunctive antibiotic domain of SNAP aims to test the effectiveness of adjunctive clindamycin compared with no adjunctive antibiotic by randomising eligible participants to one of these two groups. Future adaptations could include other adjunctive agents. Adult participants randomized to adjunctive therapy receive clindamycin 600mg three times daily intravenously (IV), or 450mg three times daily orally, for 5 days (children receive 15mg/kg per dose (maximum 600mg)). Patients with C*lostridioides difficile*-associated diarrhea of any severity are excluded. As of 31 July 2024, 3057 participants have been recruited to the SNAP platform with 2582 (84.5%) enrolled in the adjunctive antibiotic domain.

SNAP Early Oral Switch Domain

The early oral switch (EOS) domain of SNAP aims to test whether partial oral treatment of SAB is non-inferior to traditional prolonged IV treatment. Due to the high mortality and risk of occult metastatic infection associated with SAB, many clinicians understandably tend towards the most conservative treatment options. RCTs such as POET[14], OVIVA[15], and SABATO[16] have demonstrated non-inferiority of EOS in various complicated and uncomplicated *S. aureus* treatment scenarios, but for reasons of design and/or recruitment have not delivered the necessary SAB-specific evidence required to influence practice on a large scale.

Participants enrolled into SNAP are assessed for eligibility for the EOS domain at trial day 7 and day 14. The day 7 eligibility criteria identify those with uncomplicated SAB while the day 14 criteria encompass the remainder of participants (complicated SAB). If the participant is judged eligible, the randomised allocation is revealed and the patient switches to oral antibiotics or remains on IV antibiotics accordingly. The choice of oral antibiotics is at the treating clinician's discretion, although a hierarchical table of recommendations is provided which align with the allocated 'backbone antibiotic' choice. Adherence is recorded, but monitoring and support is according to sites' local practice.

As of 31 July 2024, 23% of platform participants (8% of those screened globally) have been entered into the EOS domain, about half at day 7 and half at day 14. In the day 7 (uncomplicated) group, the main reason for non-inclusion is the identification of complicated infection. In the day 14 (complicated) group, the main reasons for non-inclusion are lack of source control, persistent bacteraemia, and receipt of sufficient treatment. Aside from the EOS domain-specific inclusion/exclusion criteria, the 72-hour time limit on enrolment into the main SNAP platform excludes another pool of participants who may otherwise have been candidates for EOS. Enrolment varies across regions due to different levels of equipoise (Australia has moderate rates of enrolment), and not all sites choose to participate in the EOS domain. Paediatric enrolment has been minimal, as prolonged IV antibiotics are seen as undesirable and paediatricians will not risk randomising their young patients to this strategy. Despite these barriers, we have enrolled around 700 participants so far, more than have been enrolled into any other SAB EOS trial.

Conclusion

SNAP is now the largest trial of SAB treatment ever conducted and recruitment remains robust. Keys to success include enthusiastic international collaboration, the innovative and pragmatic trial design, and the relevance of the study questions to everyday clinical practice.

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CLINICAL RESEARCH

FROM BLING I TO BLING III

A stepwise program of research to determine clinical outcome benefits of prolonged beta-lactam antibiotic infusions in critically ill patients with sepsis and septic shock

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Sepsis affects between 47 to 79 million people worldwide every year. More than 11 million people die annually from sepsis – that is at least one death in 2.8 seconds every year.[1] In Australia, one quarter of all intensive care unit (ICU) patients have sepsis, and 1 in 4 of these patients will not survive to return home to their loved ones. Beyond unacceptable mortality rates, sepsis also imposes a significant financial burden, costing the Australian healthcare system $700 million each year. Despite modern therapeutic innovations, sepsis-related mortality remains a major problem. Optimising antibiotic therapy is the most effective strategy for managing critically ill patients with sepsis and may have the greatest impact on patient survival compared to other treatment approaches.

Patients with sepsis in the ICU may have significant pathophysiological changes that alter antibiotic pharmacokinetics.[2] Conventional dosing regimens rarely account for these alterations leading to sub-optimal antibiotic exposures and consequently, treatment failure. At the Antimicrobial Optimisation Group within the University of Queensland Centre for Clinical Research (UQCCR), we have described various strategies to optimise antibiotic dosing for critically ill patients, where empiric dosing regimens can be altered to ensure therapeutic exposures are consistently achieved.

One such strategy includes exploiting the pharmacodynamic characteristics of beta-lactam antibiotics through the use of prolonged infusions, which can either be extended (intravenous administration for 2 hours or longer) or continuous infusions (constant intravenous administration that could be administered as sequential infusions). Beta-lactam antibiotics display "time-dependent" bactericidal activity, which is optimal when the free drug concentration remains above the minimum inhibitory concentration of the infecting pathogen for at least 40% to 70% of the dosing interval (40% – 70% $fT_{>MIC}$). Use of prolonged infusions is therefore theoretically advantageous as it more

> **"...the current evidence presents a high degree of certainty for clinicians to consider prolonged infusions of beta-lactam antibiotics as a standard of care in the management of sepsis and septic shock."**

consistently achieves exposures associated with maximal bactericidal activity when compared to conventional intermittent infusion dosing. This is particularly important given that increasing clinical data suggest that critically ill patients may benefit from higher (e.g. 2 – 5 x MIC) and longer (100% $fT_{>MIC}$) beta-lactam antibiotic exposures than those described in earlier pre-clinical infection models.[2] However, there was uncertainty whether these pharmacokinetic and pharmacodynamic advantages translate into improved clinical outcomes for critically ill patients with sepsis. Therefore, under the combined leadership of Associate Professor Joel Dulhunty and Professor Jeffrey Lipman, our group undertook a stepwise research program, the Beta-Lactam Infusion Group (BLING) program (Figure 1), to determine whether prolonged infusions of beta-lactam antibiotics improve clinically important outcomes in critically ill adults with sepsis or septic shock.

The research program began in 2010 with the "proof-of-concept" BLING I randomised controlled trial (RCT).[3] BLING I (n = 60) was a multicentre, double-blind, double-dummy RCT comparing continuous versus intermittent infusions of beta-lactam antibiotics in five ICUs across Australia and Hong Kong. BLING I showed that continuous infusions of beta-lactam antibiotics achieved significant pharmacokinetic separation in $fT_{>MIC}$ (82% vs. 29%; p = 0.01) and higher clinical cure rates (70% vs. 43%; p = 0.037) compared to intermittent infusions in critically ill patients with sepsis. Our group built on this with BLING II (n = 432),[4] which was a multicentre, double-blind, double-dummy Phase 2b RCT in 25 ICUs across Australia, Hong Kong, and New Zealand. Although survival was not significantly different between the two treatment arms, there was a 1.8% to 4.4% reduction in absolute mortality measured at ICU discharge, hospital discharge, and day 90 in the continuous infusion arm compared to the intermittent infusion arm. Subsequent to BLING I and BLING II, we performed an individual patient data meta-analysis of RCTs (n = 632),[5] comparing continuous and intermittent infusions of beta-lactam antibiotics in sepsis patients and found a decreased risk of hospital mortality at day 30 favouring the continuous arm (19.6% vs. 26.3%; relative risk, 0.74; 95% confidence interval 0.56 to 1.00; p = 0.045). Given consistent clinical effects favouring continuous beta-lactam infusions in previous underpowered trials, there was sufficient justification to conduct a larger Phase 3 RCT to determine if there is a survival benefit associated with continuous infusions in critically ill patients with sepsis. Therefore, BLING III represented the final step in our structured stepwise program of research to answer this important clinical question.

BLING III (n = 7202) was an international, open-label Phase 3 RCT conducted in 104 ICUs across Australia, Belgium, France, Malaysia, New Zealand, Sweden, and United Kingdom.[6] BLING III compared continuous versus intermittent infusions of an equivalent 24-hour dose of beta-lactam antibiotics on all-cause 90-day mortality (primary outcome) in critically ill patients with sepsis. Other secondary (e.g. clinical cure at day 14 and ICU mortality) and tertiary (days alive and free of ICU stay, hospital stay, mechanical ventilation, and renal replacement therapy) outcomes were also evaluated. The mean age of patients was 60 years, and the mean APACHE II score was 20. The most common primary site of infection was pulmonary (59.5%), and 41% of patients were culture positive at the primary site of infection (Gram-negative 69% and Gram-positive 31%). Within 90 days, there was no statistically significant difference in mortality between patients who received continuous versus intermittent infusions (24.9% vs. 26.8%; absolute difference –1.9%; odds ratio, 0.91; 95% confidence interval 0.81 to 1.01; p = 0.08). The number needed to treat for continuous beta-lactam infusions to prevent one death was 50 patients. However, in the pre-specified adjusted analysis, continuous infusion was associated

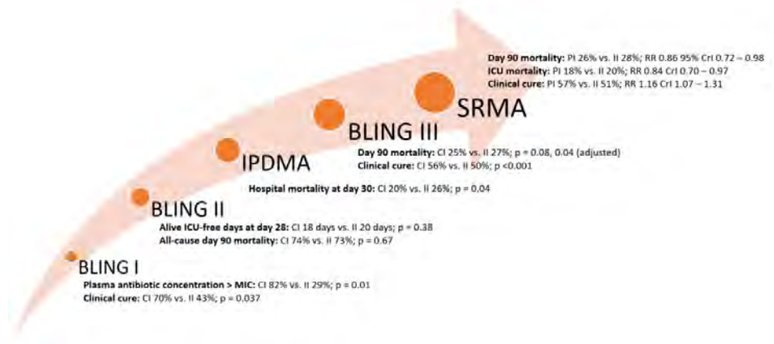

Figure 1. The BLING stepwise program of research.
Legend: CI, continuous infusion; CrI, credible interval; ICU, intensive care unit; II, intermittent infusion; IPDMA, individual patient data meta-analysis; MIC, minimum inhibitory concentration; PI, prolonged infusion; RR, relative risk; SRMA, systematic review and meta-analysis.

Prolonged infusions of beta-lactam antibiotics in critically ill patients with sepsis in the ICU						
Population	Critically ill patients with sepsis treated in the ICU					
Intervention	Prolonged infusions of beta-lactam antibiotics					
Comparison	Standard intermittent infusions of beta-lactam antibiotics					
Outcome	**Number of trials Number of participants**	**Certainty of evidence[a] (Quality of the evidence)**	**Prolonged infusion n/N (%)**	**Intermittent infusion n/N (%)**	**Absolute difference (95% CrI)**	**Risk ratio (95% CrI)**
All-cause 90-day mortality	16 trials 8989 participants	**High** ⊕⊕⊕⊕	1150/4476 (25.7)	1273/4513 (28.2)	-0.03 (-0.09 to 0.00)	0.86 (0.72 to 0.98)
ICU mortality	15 trials 8967 participants	**High** ⊕⊕⊕⊕	806/4466 (18.0)	911/4501 (20.2)	-0.03 (-0.08 to 0.0)	0.84 (0.70 to 0.97)
Clinical cure	11 trials 8276 participants	**Moderate [b]** ⊕⊕⊕⊖	2358/4125 (57.2)	2098/4151 (50.5)	0.11 (0.05 to 0.18)	1.16 (1.07 to 1.32)
Microbiological cure	4 trials 352 participants	**Very Low [c]** ⊕⊖⊖⊖	145/174 (83.3)	126/178 (70.8)	0.13 (-0.02 to 0.28)	1.18 (0.96 to 1.48)
Adverse events	4 trials 7761 participants	**Very Low [d]** ⊕⊖⊖⊖	42/3868 (1.1)	49/3893 (1.3)	-0.00 (-0.06 to 0.04)	0.89 (0.51 to 1.57)
ICU length of stay	11 trials 8911 participants	**Low [e]** ⊕⊕⊖⊖	12.5 days	13.0 days	-0.44 (-1.12 to 0.24)	-

Abbreviations: CrI, credible interval; ICU, intensive care unit

Figure 2. Grading of Recommendations, Development, and Evaluation (GRADE) summary of findings
Legend. [a] Grading of Recommendations Assessments, Development, and Evaluation (GRADE) approach specifies four levels of certainty as follows: HIGH certainty ⊕⊕⊕⊕ – we are very confident that the true effect lies close to that of the estimate of effect; MODERATE certainty ⊕⊕⊕⊖ – we are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; LOW certainty ⊕⊕⊖⊖ – our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of effect; VERY LOW certainty ⊕⊖⊖⊖ – we have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect. [b] Downgraded due to inconsistency as most studies used subjective and variable definitions of clinical cure. [c] Downgraded due to risk of bias of this outcome in the included trials, inconsistency as studies used variable definitions of microbiological cure, indirectness as microbiological cure is not directly an important patient outcome, and imprecision due to small sample size with wide credible interval. [d] Downgraded due to inconsistency as most studies used variable definitions of adverse events, indirectness as adverse events may not directly be an important patient outcome, and imprecision as the credible interval for the effect on adverse events (0.51 – 1.58) are consistent with both an appreciable benefit and appreciable harm. [e] Downgraded due to risk of bias of this outcome in the included trials, indirectness as duration of ICU stay is not directly an important patient outcome.

with a 2.2% reduction in all-cause 90-day mortality (odds ratio, 0.89; 95% confidence interval 0.79 to 0.99; p = 0.04). Clinical cure at day 14 was higher in the continuous infusion arm (55.7% vs. 50.0%; absolute difference 5.7%; odds ratio, 1.26; 95% confidence interval 1.15 to 1.38; p <0.001). Although other secondary and tertiary outcomes were not statistically different, consistent directional changes in point estimates favouring continuous infusion were observed across all outcomes.

To provide an updated summary of current evidence in light of findings from BLING III and other new trials,[7,8] we performed a systematic review and Bayesian meta-analysis[9] to assess whether prolonged infusions of beta-lactam antibiotics were associated with reduced all-cause 90-day mortality (primary outcome) compared to intermittent infusions in critically ill adults with sepsis and septic shock. Secondary outcomes included ICU mortality and clinical cure. In our review, we defined prolonged infusion as either an extended infusion or a continuous infusion. Eighteen eligible RCTs including 9108 patients were included. Compared with intermittent infusions, the probability that prolonged beta-lactam antibiotic infusions were associated with a reduced risk of 90-day mortality was 99.1% (relative risk, 0.86; 95% credible interval 0.72 to 0.98). The number needed to treat for prolonged beta-lactam antibiotic infusions to prevent one death was 26 patients. Additionally, use of prolonged beta-lactam antibiotic infusions was associated with a reduced risk of ICU mortality (relative risk, 0.84; 95% credible interval 0.70 to 0.97 and an increase in clinical cure (relative risk, 1.16; 95% credible interval 1.07 to 1.31). Based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (Figure 2), the certainty of evidence that prolonged infusions reduce all-cause 90-day mortality and ICU mortality was adjudicated as high, whereas the certainty that prolonged infusions increase clinical cure was adjudicated as moderate.

In combination with other previous meta-analyses,[5,10] the current evidence presents a high degree of certainty for clinicians to consider prolonged infusions of beta-lactam antibiotics as a standard of care in the management of sepsis and septic shock. However, several important questions remain to be investigated; what is the optimal duration of infusion when beta-lactam antibiotics are administered as prolonged infusions, and which sub-groups of sepsis and septic shock patients would benefit the most from this dosing strategy?

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Clinical Trial Update:
Trials In Surgical Antimicrobial Prophylaxis

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One in ten people in Australia undergo surgery each year and demand continues to grow (1). Surgeries, such as joint replacement surgery and cardiac surgery, lead to improvement in patient quality of life. These well recognised health and economic benefits of surgery are eroded by infections following the operation, such as wound infections (surgical site infections), pneumonia and *Clostridioides difficile* infection. These infections frequently lead to patient suffering, repeat operations, prolonged hospitalisation, prolonged antimicrobial courses, and increased mortality risk (2-5). It is estimated that over half of these infections are preventable (6).

The administration of antimicrobials at the time of surgery ('surgical antimicrobial prophylaxis') is an important infection prevention approach (2-4). The principles of antimicrobial administration in the setting of surgery align with the general principles of antimicrobial stewardship, including right indication, right drug, right dose, right route, right time and right duration (Figure 1).

Much of the practices for surgical antimicrobial prophylaxis is informed by high-quality evidence (2-4). There are, however, some key evidence gaps which can be addressed with clinical trials. This article will examine two key areas of clinical trial focus: selection of the most appropriate antimicrobials in the setting of increasingly resistant pathogens and the optimal duration of prophylaxis in high morbidity procedures.

SELECTION OF THE OPTIMAL DRUG

The safety and benefit of surgical procedures are vulnerable to emerging antimicrobial resistance (7). Joint replacement surgery is one such area impacted by increasingly resistant pathogens, in particular, methicillin resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Notably, these organisms are resistant to the first line antimicrobial recommended for surgical antimicrobial prophylaxis, cefazolin (1, 7). A number of cohort studies had suggested a potential benefit with broadening the spectrum of antimicrobial prophylaxis through adding vancomycin to cefazolin at the time of joint replacement surgery, however there was no data from randomised controlled trials to support this practice (7). Despite this, some hospitals, including Australian hospitals had adopted this approach. The Australian Surgical Antibiotic Prophylaxis (ASAP) trial was designed to investigate whether combination prophylaxis was beneficial, before widespread embedment of this approach. ASAP enrolled 4362 patients from across 11 Australian Hospitals, randomising patients without prior colonisation with methicillin resistant *Staphylococcus aureus* to cefazolin and vancomycin or cefazolin and a matched placebo. The trial found that the addition of vancomycin did not reduce the risk of infection. In secondary outcome analysis, the addition of vancomycin was associated with an *increased* risk of surgical site infection (7). Based on these findings, the routine addition of vancomycin to cefazolin is not recommended for primary joint replacement surgery in patients without known methicillin resistant *Staphylococcus aureus*. ASAP highlighted the importance of conducting randomised controlled trials to provide robust evidence on the effectiveness of interventions, and to challenge prevailing assumptions.

Figure 1. Principles of surgical antimicrobial prophylaxis administration

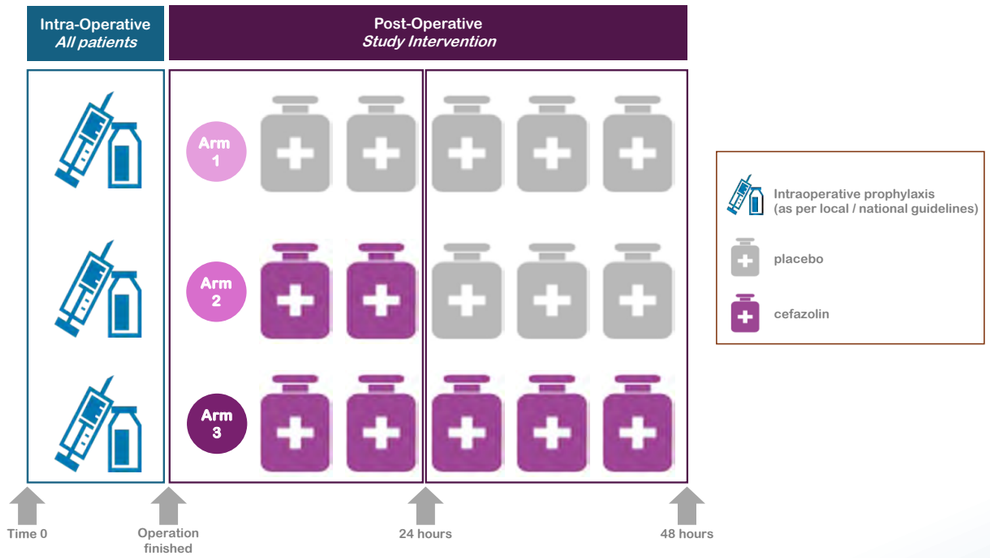

Figure 2. Principles of surgical antimicrobial prophylaxis administration

OPTIMAL DURATION OF ANTIMICROBIAL PROPHYLAXIS

Postoperative antimicrobials are not required for most surgical procedures (2-5). Extending prophylaxis for greater than 24 hours postoperatively is associated with an increased risk of patient harms including acute kidney injury, *Clostridioides difficile* infections and, conversely, an increased risk of postoperative infections due to resistant bacteria (2-5, 8). In a small number of surgery types, however, there is a potential reduction in the risk of postoperative infections when postoperative doses of antimicrobials are administered. Cardiac surgery is one such example (2, 3). Data from three randomised controlled trials in cardiac surgery have suggested the administration of postoperative doses of prophylaxis was associated with a reduction in the risk of infection, however these trials were small, heterogeneous and low-quality.

The Duration of Cardiac Antimicrobial Prophylaxis Outcomes (CALIPSO) Trial is an adaptive, double-blind, three-arm, placebo controlled, noninferiority trial comparing intraoperative only to 24 hours and, to 48 hours postoperative prophylaxis with cefazolin (Figure 2).

The trial is funded through the Medical Research Future Fund (MRFF) and conducted through the Australian and New Zealand College of Anaesthetists Clinical Trial Network (ANZCA CTN). The trial will examine the benefits and harms of postoperative doses of antimicrobial prophylaxis in 9180 patients undergoing cardiac surgery involving a median sternotomy. The trial is adaptive, meaning that there is the potential to drop an intervention arm, if there is harm identified, whilst continuing to recruit the other two arms. CALIPSO is currently recruiting across Australia and New Zealand, with international sites coming on board in 2025 (ClinicalTrials.gov NCT05447559).

THE FUTURE OF TRIALS IN SURGICAL INFECTION PREVENTION

In addition to knowledge gaps in the optimal approach to surgical antimicrobial prophylaxis, other areas of infection prevention in surgery also lack high-quality evidence to inform practice. Traditional randomised controlled trials (such as ASAP), compare a fixed number of interventions and continue to recruit participants until a prespecified sample size is reached. These trials usually take years to complete and run the potential risks of the trial been overpowered (where a finding could have been reached faster with fewer patients) or underpowered (with no definitive finding elucidated at the conclusion of the study) or futile (a study in which a true difference was never going to be identified). This adds to the expense and may reduce the impact of these trials (9). In addition, the time from reporting of study findings to translation into practice is slow with traditional designs. Adaptive platform trials are innovative trial designs that allow for the testing of multiple interventions simultaneously, with opportunities to frequently assess data throughout the trial and the ability to drop or add new interventions over time. Thereby improving the efficiency of trials and the rapidity of translation of findings into clinical practice (Figure 3) (9).

The benefits of adaptive platform trials to rapidly answer clinical questions and change the global healthcare practice have been evident during the COVID-19 pandemic (10). This methodology is ideally suited to examine multiple strategies to optimise the prevention of postoperative infections. The Perioperative Medicine Platform Trial (PROMPT) is a multicentre, embedded multifactorial adaptive platform trial for evaluating postoperative infection prevention approaches across a range of procedure types and patient groups. The trial is currently under development lead by Monash University.

CONCLUSIONS

Given the volume of surgery performed in Australia, in which many procedures will receive antimicrobial prophylaxis, this represents a high volume of antimicrobial use. There are many opportunities to examine and improve practice in this field, through the implementation of clinical trials.

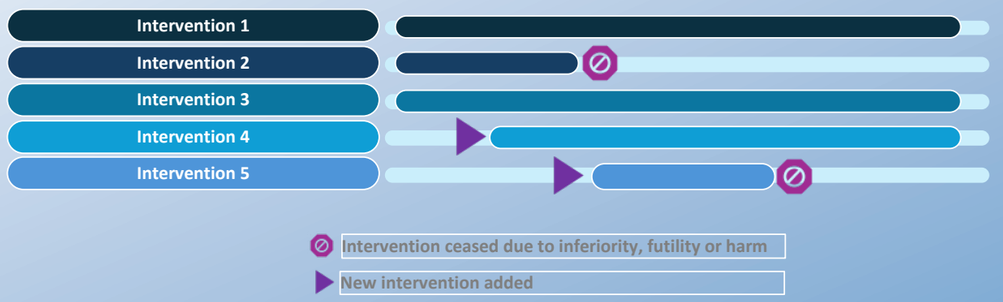

Figure 3. Principles of surgical antimicrobial prophylaxis administration

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ISSSI 2024

19th International Symposium on Staphylococci & Staphylococcal Infections

18 – 21 August 2024

Hosted by the Australian Society for Antimicrobials (ASA), the 19th International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) was recently held at the Perth Convention and Exhibition Centre. Typically held every two years, the symposium was originally scheduled to be held in 2020 but had to be delayed twice due to the COVID-19 pandemic. ISSSI, an interdisciplinary meeting, was first held in Warsaw, Poland in 1970, and has subsequently been held across the globe including Cairns in 2008 – also hosted by ASA.

The symposium attracted 179 participants and included delegates from Australia, North America, and several Asian, European, and African countries. The program spanned 3.5 days and included five plenaries, nine symposia, a proffered paper session and a poster session. Overall, 129 abstracts were accepted by the scientific committee, resulting in 22 proffered papers and 107 posters. Each symposium typically consisted of two invited speakers (one Australian and one international) and two proffered papers, providing young scientists the opportunity to present at an international meeting.

The program's plenary and symposium sessions included a variety of themes and included talks presented by key researchers including:

- Staphylococci in the Human/Animal Microbiome (Michael Otto, USA)
- Staphylococcal Genomics (Timothy Stinear, Australia)
- Controlling MRSA Outside of Hospitals (Susan Huang, USA)
- Bacteriophage Solutions for Staphylococcal Problems (Jon Iredell, Australia)
- Staphylococcal Evasion of Antimicrobial and Host Immune Attack (Anton Peleg, Australia)
- Coagulase Negative Staphylococci (Rosni Ibrahim, Malaysia and Marc Stegger, Denmark)
- Staphylococcal Pathogenesis (Ian Monk, Australia and Tracy Palmer, UK)
- Advances in Staphylococcal Surveillance (Matthew Holden, UK and Asha Bowen, Australia)
- Staphylococcal Mobile Genetic Elements (Joshua Ramsay, Australia and Jodi Lindsay, UK)
- *Staphylococcus aureus* – One Health (Henrike Krüger-Haker, Germany and Andrew Henderson, Australia)
- CA-MRSA (Margaret Ip, Hong Kong and Michael David, USA)
- *Staphylococcus aureus* and Cystic Fibrosis (Barbara Kahl, Germany and Sarath Ranganathan, Australia)
- Antimicrobials – Treatment Approaches (Steven Tong and Natasha Holmes, Australia)

Three excellent satellite symposium sessions were held during the lunch breaks:

- Pfizer Lunch – An Overview of Serious Community-Acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Infections in Australia
- European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Staphylococci and Staphylococcal Diseases (ESGS) – which included presentations by the President and Past President of ESCMID, Dr Robert Skov (Denmark) and Professor Annelies Zinkernagel (Switzerland)
- International Society of Antimicrobial Chemotherapy (ISAC) – MRSA and Infection Control – which included a presentation by the Past President of ISAC, Professor Andreas Voss (The Netherlands)

To assist young scientists to attend the symposium, five young investigator travel awards were provided to applicants who were presenting at the meeting. Each successful awardee was provided free registration, 1,000 AUD and an award certificate. The successful awardees were:

- Mia Aarris, Staten Serum Institute, Denmark
- Virginia de Lourdes Conceicao, Direccao Geral Laboratorio de Saude, Timor Leste
- Marcelino Garrine, Manhiça Health Research Centre, Mozambique
- Gi Yong Lee, University of Southern California, USA
- Kelly Peterken, The University of Auckland, New Zealand

We would also like to acknowledge the ESCMID ESGS for providing an additional two young investigator travel awards, also to the value of 1,000 AUD, which were awarded to:

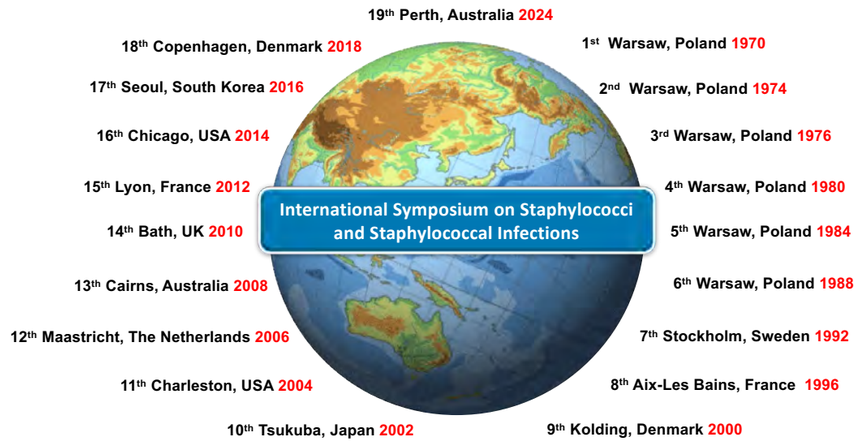
- Mariana Andrade, Universidade Nove de Lisboa, Portugal
- James Lee, University of Adelaide, Australia

In addition to the travel awards, the Australian Society for Microbiology, and the American Society for Microbiology each provided awards to the two best posters. The best poster awards, which included 250 USD and a certificate were awarded to:

- Marina Suppi, The University of Melbourne, Australia
- Freja Cecile Mikkelsen, The University of Copenhagen, Denmark

Although ASA was the host society, several societies were invited to be auspice societies of the symposium. We would like to thank the following societies for promoting the meeting over six years:

- Australasian College for infection Prevention and Control
- Australasian Society for Infectious Diseases
- The Australian Society for Microbiology
- International Society of Antimicrobial Chemotherapy



Mia Aarris
ISSSI Travel Award

Virginia de Lourdes Conceicao
ISSSI Travel Award

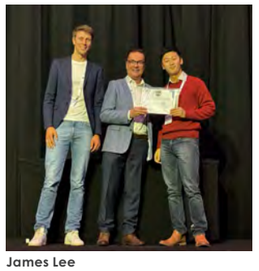
Mariana Andrade
ESGS Travel Award

Kelly Peterken
ISSSI Travel Award

Gi Tong Lee
ISSSI Travel Award

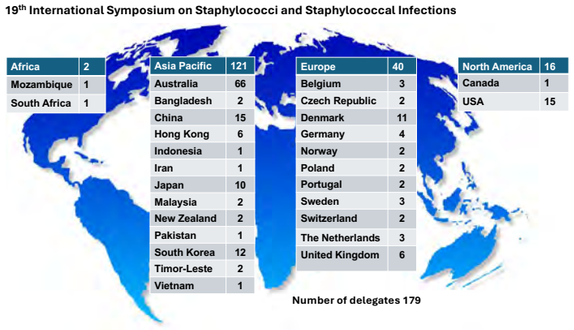
Freja Cecilie Mikkelsen
ISSSI ASM Poster Award

Marcelino Garrine
ISSSI Travel Award



James Lee
ESGS Travel Award

19th International Symposium on Staphylococci and Staphylococcal Infections



Africa	2	Asia Pacific	121	Europe	40	North America	16
Mozambique	1	Australia	66	Belgium	3	Canada	1
South Africa	1	Bangladesh	2	Czech Republic	2	USA	15
		China	15	Denmark	11		
		Hong Kong	6	Germany	4		
		Indonesia	1	Norway	2		
		Iran	1	Poland	2		
		Japan	10	Portugal	2		
		Malaysia	2	Sweden	3		
		New Zealand	2	Switzerland	2		
		Pakistan	1	The Netherlands	3		
		South Korea	12	United Kingdom	6		
		Timor-Leste	2				
		Vietnam	1				

Number of delegates 179

- Asia Pacific Society of Clinical Microbiology and Infection

In addition, we would like to give a big thank you to the meeting's generous sponsors – the meeting may not have taken place without their financial support:

- Pfizer (in particular David Grolman and Theo Mahendradatta)
- AstraZeneca (in particular Merrin Tulloch)
- Deakin University (in particular Cathy Bennett)
- Western Australia Business Events Perth

We are excited to announce many of the speakers have agreed to make their presentations available on the ASA website. The presentations can be located under the "affilitates" tab on the home page of the website (https://www.asainc.net.au/isssi-2024-presentations/). The Australian poster presenters have also made their abstracts and posters available to ASA, and are featured in this edition of the ASA newsletter.

The 19th ISSSI was enjoyed by all those who attended, and we thank all the delegates, many who had to travel very long distances. Their contribution, whether as a speaker, poster presenter, or attendee made the symposium a great success. The insights shared and the connections made during ISSSI were truly inspiring, and their attendance created an event that was both educational and impactful. We look forward to seeing you all at the 20th ISSSI which will be held in Banff, Canada in September 2026.

Geoffrey Coombs
Shakeel Mowlaboccus
Murdoch University | Western Australia

ISSSI 2024

Original research conference posters

Staphylococcus aureus **strains with a negative coagulase tube test are associated with Staphylocoagulase genotypes**

Carly L. Botheras, Dieter Bulach, Eugene Athan

The Australian Group on Antimicrobial Resistance (AGAR) 2022 Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP)

D. A. Daley, G. W. Coombs, P. Shoby, and S. Mowlaboccus, on behalf of the Australian Group on Antimicrobial Resistance

The Australian Group on Antimicrobial Resistance (AGAR) trend data 2013 - 2022 from the Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP)

D. A. Daley, G. W. Coombs, P. Shoby, and S. Mowlaboccus, on behalf of the Australian Group on Antimicrobial Resistance

Isolation of bacteriophages against methicillin-resistant *Staphylococcus pseudintermedius* in dogs

Makayla Donovan, Kate Worthing, Carola Venturini

Bioinformatic identification of genes associated with resistance in *Staphylococcus saprophyticus*

Paulina Hall, Johannes Zuegg, Mark Blaskovich

Media-dependent antibiotic resistance in *Staphylococcus aureus*

Bonnie L. Hyatt, M. Kalindu D. Rodrigo, Timothy C. Barnett

A novel mechanism in the switch to a stable small colony variant of *Staphylococcus aureus* associated with dysfunctional glucose metabolism

James Lee, Miguel Carda Sarah Vreugde, Matipaishe Mashayamombe, Joseph Dawson, Robert Fitridge, Alex Mira, Peter S. Zilm, Stephen P. Kidd

Healthcare-associated *Staphylococcus aureus* bloodstream infections associated with peripheral intravenous cannulae in Western Australian hospitals, 2019 to 2023

Khui Hung (Claire) Lee, Liana Varrone, Inutu Kashina, Olivia Kamau, Lisa Nicolaou, Melanie Trainor, Rebecca Hogan, Rebecca McCann

The diagnostic utility of different imaging modalities for paediatric *Staphylococcus aureus* bacteraemia

Rachel Rachel, Asha C. Bowen, Anita J. Campbell

Can commensal *Staphylococcus felis* kill methicillin-resistant *Staphylococcus pseudintermedius in-vitro*?

Isabella Singarayar, Dr Kate Worthing

PknB kinase directly controls the WalKR system and modulates cell wall antibiotic resistance in *Staphylococcus aureus*

Marina Suppi, Stephanie Tan, Ian R. Monk, Liam Sharkey, Aakash Natarajan, Katharine Myler, Sheila Marie Pimentel-Elardo, Jan V. T. Falguera, Timothy P. Stinear, Sacha J. Pidot, Justin R. Nodwell

Diversity of MRSA clones reported in Western Australian

H-L Tan, G. Coombs, S. Mowlaboccus, O. Robinson

Genomic epidemiology of clonal complex 1 *Staphylococcus aureus* from remote communities in Western Australia

N. W. T. Yee, S. Mowlaboccus, M. Stegger, S. Baig, H-L. Tan, G. W. Coombs

STAPHYLOCOCCUS AUREUS STRAINS WITH A NEGATIVE COAGULASE TUBE TEST ARE ASSOCIATED WITH STAPHYLOCOAGULASE GENOTYPES

Dr. Carly Botheras[1,2*], Dr. Dieter Bulach[3], Professor Eugene Athan[1,2,4]

[1]Centre for Innovation in Infectious Disease and Immunology Research, School of Medicine, Deakin University, Victoria, Australia [2]Institute for Innovation in Physical and Mental Health and Clinical Translation, Deakin University, Victoria, Australia, [3]Department of Microbiology and Immunology, The University of Melbourne, Victoria, Australia, [4]Department of Infectious Disease, University Hospital Geelong, Barwon Health, Victoria, Australia * Presenting Author: carly.botheras@deakin.edu.au

Introduction

In 2019 alone, *S. aureus* caused over one million deaths globally (1).
Conversely, many coagulase-negative species are considered less virulent and are frequent contaminants.
Therefore, the identification of coagulant ability is a priority in diagnostic laboratories with suspected staphylococcal species.
The coagulase tube test is still used in many countries as either an initial test or the primary identification method.
Reports of coagulase tube test negative *S. aureus* ranges between 2%-16% (2).
A possible explanation is the genetic variation of the staphylocoagulase gene (*coa*).
In this study, we investigated whether there was an association between *coa* genotypes and negative results in the coagulase tube test.

Coagulase Tube Test

Isolates
One-hundred and twenty-two *S. aureus* clinical isolates collected from human bloodstream infections in the Barwon South-West region of Victoria, Australia were used for this study.

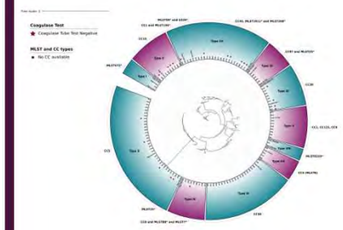
Coagulase and accessory gene regulator production detection
The 122 isolates were assessed using the coagulase tube test as per manufacturer's instructions (3), and accessory gene regulator function was assessed using the CAMP assay (4). These were performed in biological triplicate.

Results
Negative strains were present in 18.9% of the cohort.
Accessory gene regulator function did not appear to affect the coagulase test results (OR: 0.50, 95% CI: [0.14, 1.82], p=0.290; binomial logistic regression, SPSS V29.0).

A single colony was inoculated into commercially prepared rabbit plasma, the tube was incubated at 37 degrees Celsius in a heat block, and check for signs of coagulation at hours 1 through 4 and then at hour 24 and 48. Each isolate assessed in biological triplicate.

Detecting Genotypes of *staphylocoagulase*

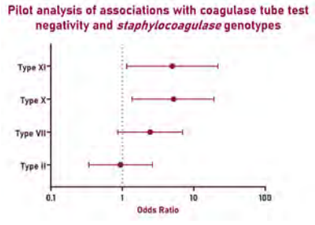
Assessing the *staphylocoagulase* Genotypes of clinical *S. aureus* isolates
Sequencing of each of the 122 isolates' genomes was performed via an illumina mi-seq using described methods (5). The Nullarbor bioinformatic pipeline (6) (V2.0.20181015) was used to perform quality control, the assembly, & genome annotation (readsets available at NCBI, accession number PRJNA611667).



The *coa* sequence was extracted and compared to the reference sequences of each *coa* genotypes (7) using ClustalW (V2.1). Phylogenetic trees were prepared on FastTree (V2.1.10) and further visualised on Interactive Tree of Life (V6.9.1).

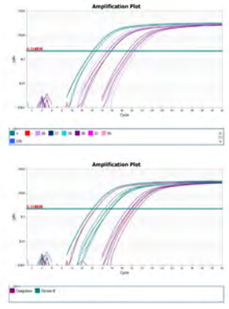
Similar to prior studies, the staphylocoagulase genotypes were observed to be conserved across Multi-locus sequence types, which have been concatenated into clonal complex (CC) types were possible in the figure.

Associations between genotypes and coagulase production



Coagulase tube test and *staphylocoagulase* genotypes
coa genotypes with negative coagulase tube test results and had 10 or more representative isolates were analysed using a univariable binomial logistic regression (SPSS V29.0, visually represented using GraphPad Prism V9.4.1).
Genotypes X and XI were found to be significantly associated with negative coagulase tube results.

Expression of *staphylocoagulase*



staphylocoagulase **Expression**
A small subset of isolates were chosen as representative isolates from multiple genotypes, and from coagulase test results to represent both positive and negative strains.

Using the sub-selection of isolates, reverse transcription real time quantitative polymerase chain reaction (RT-qPCR), 3hours into growth (8), was performed to measure expression of the *coa* gene. This was performed in technical triplicate and a subset of results depicted in the figures to the left. Seen to the left is a representation of some of the isolates by sample (above) and by target (below).

Initially designed to measure *coa* expression in comparison to the *gyrase B* housekeeping gene, the results of the triplicate was too discordant for comparison. However, the fact that there was expression present at all, suggests expression is not contributing to the results of the coagulase tube test.

Discussion points

- 18.9% of this cohort tested negative in the coagulase tube test, highlighting a potential risk of false negatives of *S. aureus* identification to note for laboratory scientists.
- At the genetic level, all isolates had the *staphylocoagulase* gene present. There was only one isolate that had a major deletion in the conserved region that could have theoretically affected coagulase tube test positivity. Expression work in a subset of isolates, suggests that it is not an expression deficit either.
- Types X and XI may be diverging away from rabbit plasma, making this quick and easy test complex, both types are conserved to CC 15 and 20 strains.
- The Type X genotype has been recognised to be 98.5% similar to fibronectin binding protein A, suggesting a potential evolutionary change to a different role in these isolates (8).
- *S. aureus* can infect other animals, as seen by Type IX, which has only been in livestock associated strains, and not present in this cohort.
- Interestingly a 2011 study investigated isolates collected from human, bovine and dog and identified that dog plasma was superior to rabbit plasma, and although no false-negative occurred in their samples, the initial isolates were identified using the rabbit plasma (9).
- While not a clinically significant variation, the fact that *S. aureus* strains may test negative in the coagulase test is still an important observation and it may be more relevant where these strains are more prominent.

ACKNOWLEDGEMENTS

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The Australian Group on Antimicrobial Resistance (AGAR)
Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP) 2022

D. A. Daley[1,3], G. W. Coombs[1,2,3] P. Shoby[2] and S. Mowlaboccus[1,2,3] on behalf of the Australian Group on Antimicrobial Resistance

[1]The Australian Group on Antimicrobial Resistance, Fiona Stanley Hospital, WA, [2]Antimicrobial Resistance and Infectious Diseases Laboratory (AMRID), Murdoch University, WA, [3]Department of Microbiology, PathWest Laboratory Medicine, Fiona Stanley Hospital, WA.

INTRODUCTION

In 2022, 33 institutions across Australia servicing 55 hospitals participated in the AGAR Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP). The primary objective of ASSOP 2022 was to determine the proportion of *S. aureus* bacteraemia (SAB) isolates in Australia that exhibited antimicrobial resistance, with particular emphasis on susceptibility to methicillin, and to characterize the molecular epidemiology of the methicillin-resistant *S. aureus* (MRSA).

METHODS

Isolates

From 1 January to 31 December 2022 the 33 participating laboratories collected all *S. aureus* isolated from blood cultures (excluding duplicates within a 14-day period). Data were collected on age, sex, date of admission and discharge and mortality at 7 and 30 days post blood culture collection.

Susceptibility testing

Isolates were identified by the participating laboratories and antimicrobial susceptibility testing was performed using the Vitek® 2 (bioMérieux, France) or the BD Phoenix™ (Becton Dickinson, USA) automated microbiology systems. European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were utilised for interpretation.

Whole genome sequencing testing

Whole genome sequencing (WGS) was performed on MRSA isolates at the AMRID laboratory, Murdoch University, WA.

RESULTS

- 3,214 unique episodes of SAB were identified.
- The mean patient age was 59 years. 66.6% of patients were male.
- 77.5% of episodes were community-onset.
- All-cause mortality at 30 days was 17.5% (95%CI 16.1-19.0). There were no significant difference in mortality between hospital-onset and community-onset SAB (19.5% and 17.0% respectively) (P = 0.16). There was however between MRSA and methicillin-susceptible *S. aureus* (MSSA) bacteraemia (21.4% and 16.8% respectively) (P = 0.02).
- Osteomyelitis/septic arthritis (20.8%) and skin/skin structure (19.7%) were the most common principal clinical manifestations.

MSSA

- With the exception of the β-lactams and erythromycin, antimicrobial resistance in MSSA was rare (Table 1). Resistance was not detected for daptomycin, vancomycin, linezolid or teicoplanin by CLSI criteria.

MRSA

- 15.0% (481) *S. aureus* were methicillin resistant. Comparison with EARS-Net and WHO CAESAR data is shown in Figure 1. One MRSA isolate with a daptomycin MIC of 1.5 mg/L harboured the A302V MprF and A23V Cls2 mutations. Resistance was not detected for vancomycin, linezolid or teicoplanin by CLSI criteria.
- 449 (93.3%) MRSA were available for typing by WGS. Overall 13.6% and 86.4% were classified as healthcare-associated (HA) and community-associated (CA) clones respectively (Figure 2). 52.5% and 78.4% of HA-MRSA and CA-MRSA respectively were community-onset.

RESULTS (continued)

Table 1. *Staphylococcus aureus* susceptibility data, 2022

	Methicillin-resistant					Methicillin-susceptible				
		CLSI		EUCAST			CLSI		EUCAST	
Antimicrobial	Number	% I	% R	% S-IE	% R	Number	% I	% R	% S-IE	% R
Benzylpenicillin*	480	–†	100.0	–†	100.0	2,719	–†	74.9	–†	74.9
Ciprofloxacin	479	1.3	29.4	69.3	30.7	2,724	0.7	2.2	97.0	3.0
Clindamycin§	479	0.0	23.0	0.0	24.2	2,722	0.0	10.8	0.0	11.5
Erythromycin	478	17.6	28.7	–†	29.7	2,669	28.2	13.2	–†	13.9
Gentamicin	479	3.3	5.8	–†	11.3	2,724	0.8	0.7	–†	3.5
Mupirocin (high-level)	346	–†	2.0	–†	2.0	2,096	–†	1.2	–†	1.2
Rifampicin	479	0.0	1.0	–#	1.5	2,721	0.1	0.3	–#	0.6
Tetracycline/doxycycline	479	0.0	10.9	–†	13.2	2,720	0.1	2.7	–†	3.5
Trimethoprim–sulfamethoxazole	478	0.4	1.7	0.4	1.7	2,723	0.2	0.3	0.1	0.4

I = intermediate; R = resistant; S-IE = susceptible, increased exposure

* = β-lactamase adjusted; † = no category defined; § = constitutive + inducible; # = rifampicin concentration range on some cards restricts category interpretation

Figure 1. Methicillin-resistant *Staphylococcus aureus*, international comparisons, WHO European region and Australia, 2022

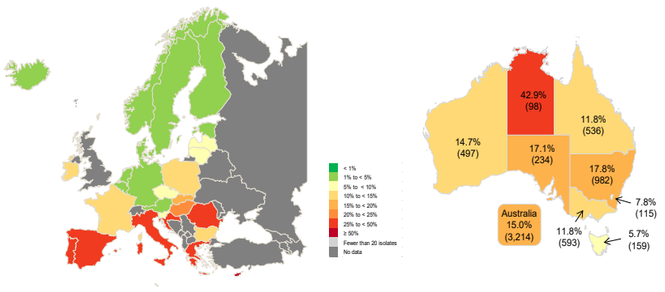


Figure 2. HA- and CA-associated MRSA clones, by state and territory, 2022

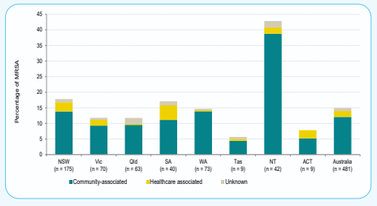
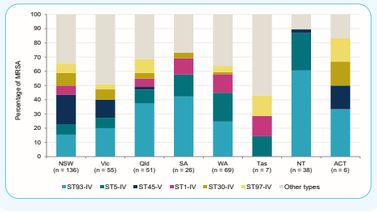


Figure 3. HA-associated MRSA, by state and territory, 2022

Figure 4. CA-associated MRSA, by state and territory, 2022



- Two HA-MRSA clones were identified, ST22-IV [2B] (EMRSA-15) and ST239-III [3A] (Aus-2/3 EMRSA) (Figure 3).
- Although polyclonal (64 clones), 71.9% of CA-MRSA clones were classified into eight major sequence types (ST): ST93-IV, ST5-IV, ST45-V, ST1-IV, ST30-IV, ST97-IV, ST953-V and ST8-IV (Figure 4).
- Overall 166 (37.0%) of MRSA were PVL positive, all of which were CA-MRSA.

CONCLUSIONS

- ASSOP 2022 has demonstrated antimicrobial resistance in SAB in Australia remains a significant problem and is associated with a high mortality. This may be due, in part, to the high prevalence of methicillin-resistant SAB in Australia.
- MRSA must remain a public health priority and continuous surveillance of SAB and its outcomes and the implementation of comprehensive MRSA strategies targeting hospitals and long-term care facilities are essential.

Acknowledgements

We wish to thank the staff of the AGAR laboratories for the collection of data and isolates.

The AGAR Surveillance Outcome Programs are funded by the Australian Government Department of Health and Aged Care.

AUSTRALIAN GROUP on ANTIMICROBIAL RESISTANCE

Murdoch University

The Australian Group on Antimicrobial Resistance (AGAR)
Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP) 2013–2022

D. A. Daley[1,3], G. W. Coombs[1,2,3] P. Shoby[2] and S. Mowlaboccus[1,2,3] on behalf of the Australian Group on Antimicrobial Resistance

[1]The Australian Group on Antimicrobial Resistance, Fiona Stanley Hospital, WA, [2]Antimicrobial Resistance and Infectious Diseases Laboratory (AMRID), Murdoch University, WA, [3]Department of Microbiology, PathWest Laboratory Medicine, Fiona Stanley Hospital, WA.

INTRODUCTION

In 2013, AGAR began the Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP). The objectives were to determine the proportion of *S. aureus* bacteraemia isolates in Australia that exhibited antimicrobial resistance in particular to methicillin, and to characterize the molecular epidemiology of the methicillin resistant isolates.

METHODS

Isolates

Participating laboratories collected all *S. aureus* isolated from blood cultures (excluding duplicates within a 14-day period).

Susceptibility testing

Isolates were identified by the participating laboratories and antimicrobial susceptibility testing was performed using the Vitek® 2 (bioMérieux, France) or the BD Phoenix™ (Becton Dickinson, USA) automated microbiology systems. EUCAST breakpoints were utilised for interpretation.

Whole genome sequencing

Whole genome sequencing (WGS) was performed on methicillin-resistant *S. aureus* (MRSA) isolates by the AMRID laboratory at Murdoch University, WA.

RESULTS

- In the 10-year period 2013 to 2022, 26,484 *S. aureus* bacteraemia episodes were reported. Of these 17.7% (4,689) were methicillin resistant.

MSSA

- Resistance rates for key antimicrobials are shown in Figure 1. There has been significant decreasing 5-year trends in resistance rates to penicillin (χ^2 for trend P = 0.0033) and fusidic acid (χ^2 for trend P = 0.0491).
- Over the past 5-years, significant increasing trends have been observed in resistance rates to erythromycin (χ^2 for trend P = 0.0216), clindamycin (χ^2 for trend P = 0.0030), and gentamicin (χ^2 for trend P < 0.0001).

MRSA

- Since 2016, the proportion of *S. aureus* that was methicillin-resistant began to decline nationally, although there were notable variations at state and territory level (Figure 2). From 2018 to 2022, there was a significantly decreasing trend in MRSA in Australia χ^2 for trend P < 0.0242), notably in WA (χ^2 for trend P = 0.0074).
- Resistance rates for key antimicrobials are shown in Figure 3. Significant decreases have been observed in most key antimicrobials. This is largely due to the significant decrease in healthcare-associated MRSA clones in particular the multi-resistant ST239-III (Figures 4 and 5).
- Although numbers and diversity of CA-MRSA have increased, the most common sequence types have remained fairly stable with the exception of the increase seen in ST93-IV (Figure 6).

CONCLUSIONS

- AGAR surveillance remains core to Australia's response to the problem of increasing AMR. AGAR data contribute to understanding AMR in Australian human health settings, and to informing the national response to AMR.
- AGAR contributes internationally through annual contribution of data on *S. aureus* from blood to the World Health Organization (WHO) Global Antimicrobial Resistance and Use Surveillance System (GLASS).

Figure 1. Methicillin-susceptible *Staphylococcus aureus*, resistance to key antimicrobials, 2013–2022

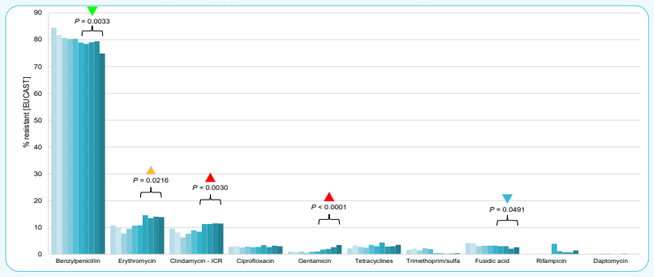


Figure 2. Proportion of methicillin-resistant *Staphylococcus aureus*, by state and territory, 2013–2022

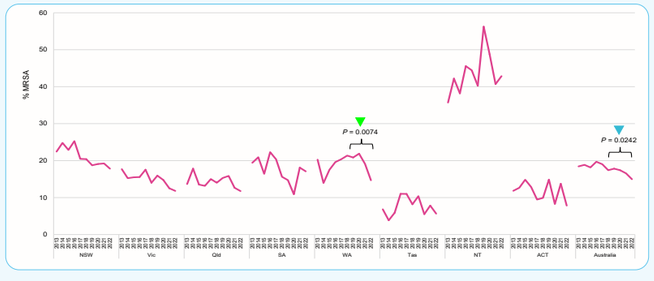


Figure 3. Methicillin-resistant *Staphylococcus aureus*, resistance to key antimicrobials, 2013–2022

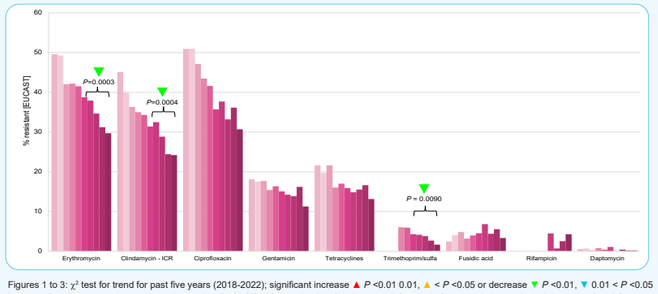


Figure 4. Healthcare- and community-associated MRSA, 2013–2022

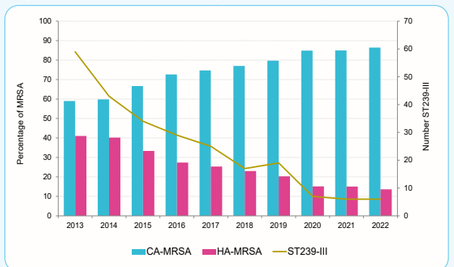


Figure 5. Healthcare-associated MRSA sequence types, 2013–2022

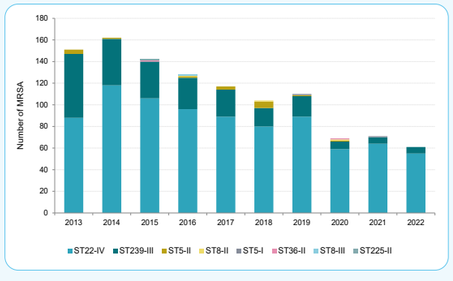
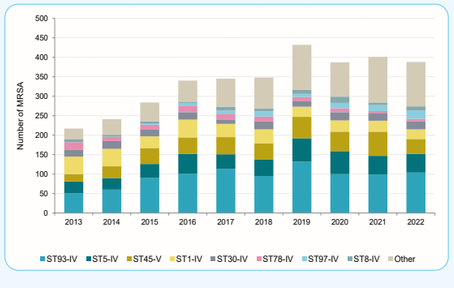


Figure 6. Community-associated MRSA sequence types, 2013–2022



Figures 1 to 3: χ^2 test for trend for past five years (2018-2022); significant increase ▲ P <0.01 0.01, ▲ < P <0.05 or decrease ▼ P <0.01, ▼ 0.01 < P <0.05

Notes: 1. Percentage resistance determined using EUCAST 2023 breakpoints for all years. 2. Data only from hospitals consistently reporting for all five years were included.

Acknowledgements

We wish to thank the staff of the AGAR laboratories for the collection of data and isolates.

The AGAR Surveillance Outcome Programs are funded by the Australian Government Department of Health and Aged Care.

AUSTRALIAN GROUP on ANTIMICROBIAL RESISTANCE

Murdoch University

Isolation of bacteriophages against methicillin-resistant *Staphylococcus pseudintermedius* in dogs

Makayla Donovan,[1] Tina Baxter,[2] Kate Worthing,[2, 3*] Carola Venturini [2, 3*]

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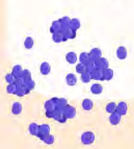
Staphylococcus pseudintermedius

Staphylococcus pseudintermedius is a significant opportunistic bacterial pathogen of dogs prevalent within a variety of infections, including pyoderma (1). It is also part of a dog's normal microflora of skin and mucosal membranes, commonly found in the perineum, mouth, nose and groin of healthy dogs (2).



Deep canine pyoderma from MRSP (3)

Introduction

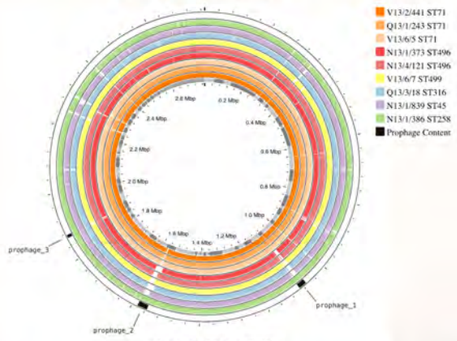
Antimicrobial Resistance

The rising prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) in veterinary medicine creates difficulties in treatment with standard antimicrobials. Ten to 20% of *S. pseudintermedius* infections in Australian dogs are MRSP (4) and an increasing proportion of these are extensively drug resistant. Several dominant clones exist but ST71 is the most widespread.

Phage Research

Bacteriophages (phages) are highly species-specific innate viral predators of bacterial pathogens that can eliminate bacteria without adverse impacts on animal or human cells (5,6). The current research on phages and their lytic properties showcases their potential for treatment, particularly against multi-drug resistant bacterial pathogens (7). However, there is minimal research into phages against MRSP in dogs.

MRSP Genomes



N13/4/21 ST64 MRSP

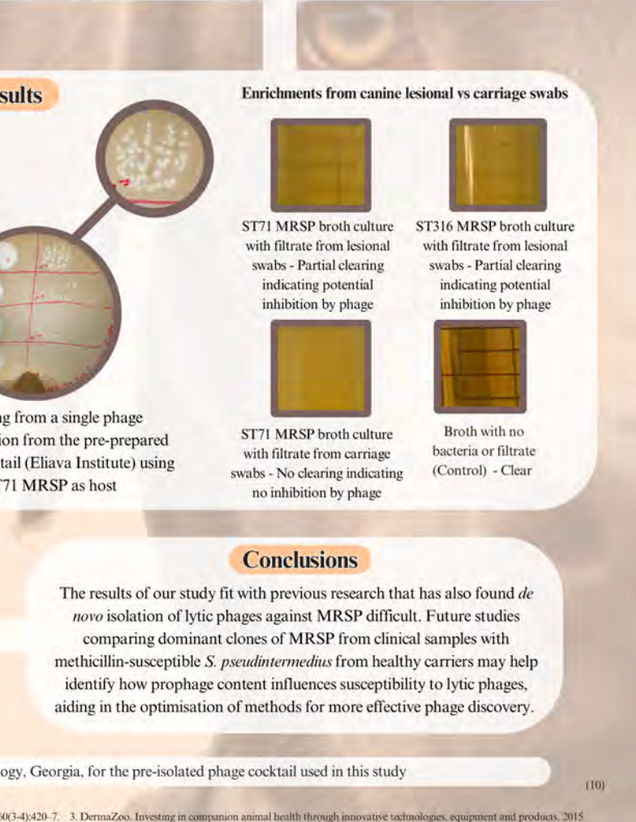
Figure 1: Comparative analysis (Proksee: 8) of MRSP genomes, highlighting differences in prophage content

Aims and Methods

Aims: To isolate and characterise lytic phages against MRSP from environmental and clinical sources

Methods:

1. Filter environmental and clinical samples
2. Enrichments: Co-incubation of target bacteria and phage filtrate sample
3. Filter enrichments
4. Double layer agar assays with target bacteria and phage filtrate sample
5. Single phage purification via plaque picking
6. Double layer agar assays to amplify pure phage culture



Made in BioRender (9)

Lytic Phage Isolated ✓ No Lytic Phage Isolated ✗ Preliminary Findings ?

Environmental and Clinical Samples Tested

Environmental

- Waste-water from a sewage treatment plant ✗
- Seafood effluent ✗

Clinical

- Dog nasal swabs ✗
- Cat and pig faecal samples ✗
- Dog nose, mouth and perineum (carriage) swabs ✗
- Dog skin lesion swabs ?

Amplification of pre-isolated phage from clinical cocktail (Eliava Institute) ✓

Results

Titering from a single phage amplification from the pre-prepared phage cocktail (Eliava Institute) using ST71 MRSP as host

Enrichments from canine lesional vs carriage swabs

ST71 MRSP broth culture with filtrate from lesional swabs - Partial clearing indicating potential inhibition by phage

ST316 MRSP broth culture with filtrate from lesional swabs - Partial clearing indicating potential inhibition by phage

ST71 MRSP broth culture with filtrate from carriage swabs - No clearing indicating no inhibition by phage

Broth with no bacteria or filtrate (Control) - Clear

Key Findings

- No lytic phages against MRSP isolated from environmental and clinical samples to date
- One putative lytic phage from Eliava Institute showed inhibition of one strain of ST71
- Half (5/10) of the surveyed MRSP genomes had at least 3 complete prophages

Conclusions

The results of our study fit with previous research that has also found *de novo* isolation of lytic phages against MRSP difficult. Future studies comparing dominant clones of MRSP from clinical samples with methicillin-susceptible *S. pseudintermedius* from healthy carriers may help identify how prophage content influences susceptibility to lytic phages, aiding in the optimisation of methods for more effective phage discovery.

Acknowledgments: George Eliava Institute of Bacteriophages, Microbiology and Virology, Georgia, for the pre-isolated phage cocktail used in this study

(10)

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Bioinformatic identification of genes associated with resistance in *Staphylococcus saprophyticus*

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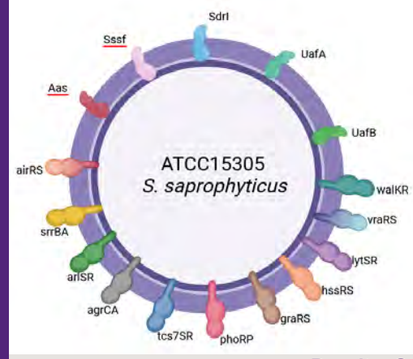
Centre for Superbug Solutions, The University of Queensland

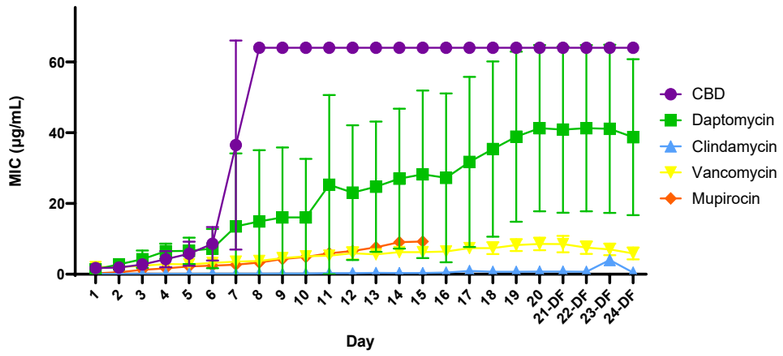
B La S K O GROUP

Background and workflow

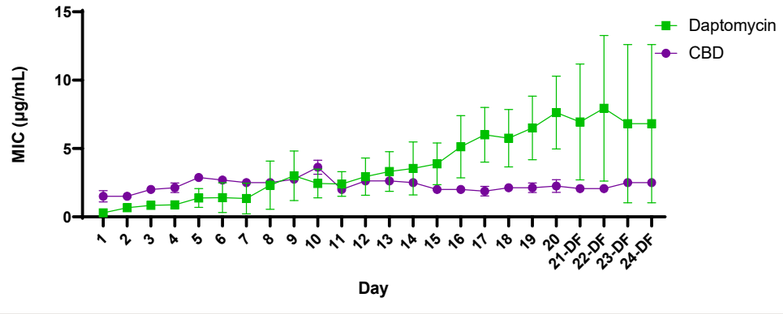
- Unlike *S. aureus, S. saprophyticus* has not acquired many resistance genes.
- Despite lack of resistance genes, 20-day resistance selection studies with *S. saprophyticus* lead to remarkably high levels of resistance for common antibiotics compared to *S. aureus*.[1]
- *S. saprophyticus* has several cell membrane adhesins required for colonisation of the urinary tract (figure 1.) and it has 11 two component signal transduction systems which are common with *S. aureus*.[2]
- One of the compounds tested is cannabidiol (CBD), which we have previously reported is an excellent Gram-positive antibiotic, with minimal resistance seen in the *S. aureus* 20-day study. We made a fortuitous discovery of *S. saprophyticus* in the *S. aureus* study.
- This project aims to investigating the genetic changes accompanying the development of resistance in *S. saprophyticus.*



S. saprophyticus serial passage study



S. aureus serial passage



Results- Genetic variants arising from 20-day antibiotic treatment

Table 1. Variants arising from Day 20 cannabidiol treatment

SNP type	Gene	Protein	Protein function	Effect of SNP on protein function
Frameshift – Glu130fs	*atpF*	ATP synthase subunit b	Connects the cytoplasmic F1 region to the transmembrane F0 region	deleterious
Frameshift – Lys7fs	*atpH*	ATP synthase subunit delta	Connects the subunit b tail to αβ ring.	under analysis
Frameshift – Val36fs	*atpB*	ATP synthase subunit a	Involved in proton conduction	under analysis
Missense – Val53Alla	*atpE*	ATP synthase subunit c	Membrane bound c-ring	deleterious
Frameshift – Met17fs	*atpG*	ATP synthase gamma chain	Part of the cytoplasmic F1 region	under analysis
Frameshift – Thr430fs	*atpD*	ATP synthase subunit beta	Part of cytoplasmic ring	deleterious
Frameshift – Val378fs	*atpA*	ATP synthase subunit alpha	Together with β forms the cytoplasmic ring	deleterious

Table 2. Variants arising from Day 20 daptomycin treatment

SNP type	Gene	Protein	Protein function	Effect of SNP on protein function
Missense - Leu137Ser	*walK*	Sensor protein kinase WalK	Part of the WalK/R system, regulates cell wall metabolism genes	Under analysis
Missense - Ser41Pro	*cls_2*	Cardiolipin synthase	Catalyses the synthesis of cardiolipin	deleterious
Missense – Ser65Pro	*vraS*	Sensor protein VraS	Regulator of cell wall homeostasis	neutral
Missense-Pro314Leu	*mprF*	Phosphatidylglycerol lysyltransferase	Required for lysinylation of phosphatidylglycerol	deleterious

Summary

- The variants identified in response to daptomycin correspond to the genes identified in the literature. Further investigation is needed to understand how these variants contribute to daptomycin resistance.
- The ATP synthase variants, together with the involvement of menaquinone in susceptibility to CBD, could suggest that its activity is through inhibition of the electron transport chain, however further studies are needed to confirm this.
- Furthermore, this research will aid in the annotation of the genome and resistome of *S. saprophyticus.*
- Aid in the understanding of how non-emergent bacteria can acquire antibiotic resistance through induced genetic mutations.

Future directions

- Complementation studies and GWAS to confirm the casual variants identified in the preliminary study.
- Setting up a statistical method for assessing gene variant significance/ frequency of mutation.
- RNA sequencing of selected *S. saprophyticus* CBD serial passage samples.
- Currently we are working on analysing the pangenome of *S. saprophyticus and* further analysis on location of variants in the identified genetic regions.
- The CBD serial passage of *S. saprophyticus* produced variable results, and further analysis is required to identify the significance of these variants in relation to the mechanism of action of CBD

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Acknowledgements

Paulina Hall would like to acknowledge the Institute for Molecular Bioscience Global Challenges program and the Blaskovich group on the opportunity to undertake this research.

THE UNIVERSITY OF QUEENSLAND AUSTRALIA
CREATE CHANGE

Media-Dependent Antibiotic Resistance in *Staphylococcus aureus*

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Background

- Antimicrobial resistance is predicted to kill 10 million people per year by 2050
- Effective and accurate antibiotic susceptibility tests (AST) are required to reduce clinical treatment failure, including for common pathogens like *Staphylococcus aureus*.
- Media-dependent antibiotic resistance can produce false susceptibility results depending on the composition of Mueller Hinton Agar (MHA) (1-3).

Aims

- To determine whether *S. aureus* displays media-dependent resistance across five different brands of MHA against folate-pathway targeting antibiotics.
- To identify which metabolites can induce sulfamethoxazole resistance in a normally sensitive *S. aureus* strain.

Results

1. Variable antibiotic susceptibility results are obtained with different brands of MHA.

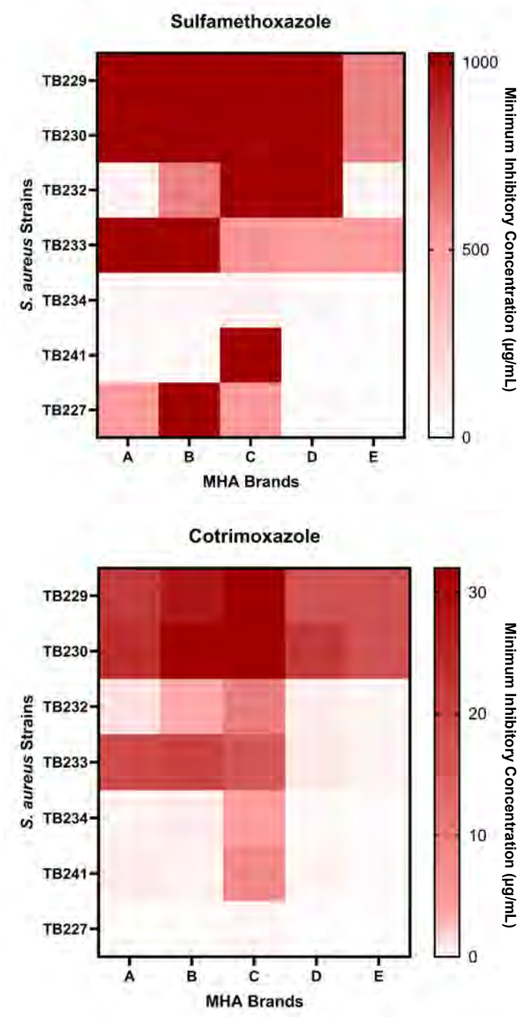

Fig 1: Media-dependent antibiotic resistance was observed across the five brands of MHA. White indicates a sensitive Minimum Inhibitory Concentration (MIC) (µg/mL) and red indicates a resistant MIC (µg/mL). MICs were determined by Epsilometer test and Kirby Bauer disk diffusion AST methods. MIC breakpoints are determined according to EUCAST breakpoints (version 13).

2. Individual metabolites can rescue *S. aureus* from an inhibitory concentration of sulfamethoxazole.

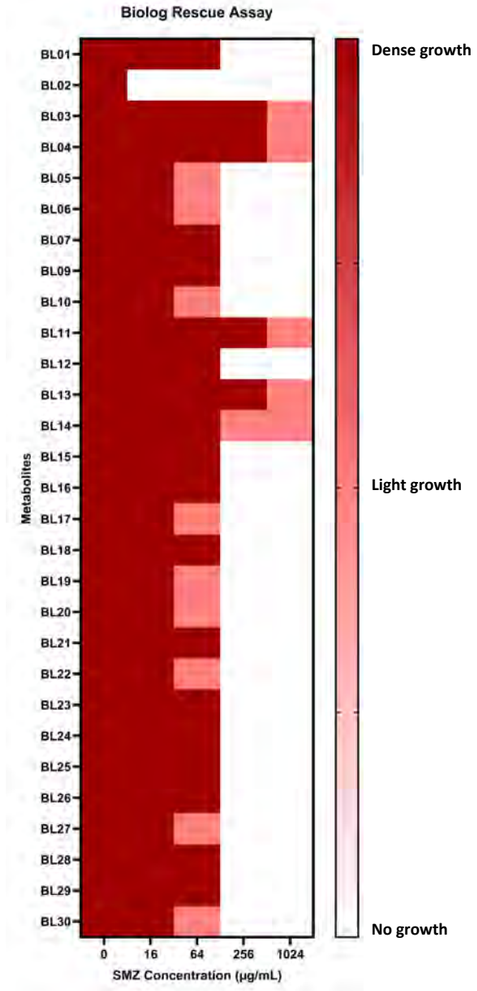

Fig 2: Biolog Microbial Phenotype Microarray plates were used to perform a metabolite rescue assay with *S. aureus* strain TB241. A total of 760 metabolites were tested against five concentrations of sulfamethoxazole (SMZ) to determine to what extent each metabolite could rescue TB241 (if at all). A total of 760 metabolites were screened with representative results for 30 metabolites shown. White indicates no growth, pink indicates light growth and red indicates dense growth.

Conclusions

- Variations in composition of MHA from different brands can substantially alter AST results.
- Phenotypic ASTs may not always reliably predict *in vivo* susceptibility of bacteria to a specific antibiotic because the composition of MHA does not necessarily reflect the *in vivo* environment (i.e. the susceptibility result obtained *in vitro* is not necessarily the same for *in vivo* susceptibility)
- Future studies should investigate the minimum concentration of each metabolite that is required to induce resistance.

Acknowledgements and References

This work was supported by the Western Australia Future Health Research and Innovation Fund through the Translation Fellowships 2021 Program.

We acknowledge and thank AGAR, Geoff Combes and Shakeel Mowlaboccus for providing the strains used in this project.

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2. Ahman et al., 2022, *Clin Microbiol Infect*
3. Rodrigo et al., 2022. *Nat Commun*

A novel mechanism in the switch to a stable Small Colony Variant of *Staphylococcus aureus* associated with dysfunctional glucose metabolism

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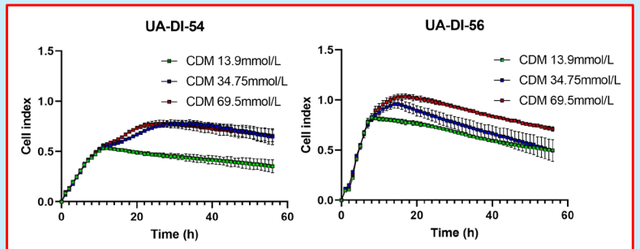
BACKGROUND

Small Colony Variants: a persistent cell type that confers antibiotic tolerance and evades the immune response

We have isolated a **stable small colony variant (sSCV)**, UA-DI-54, and a **non-stable small colony variant**, UA-DI-56, from the ulcer of a diabetic foot infection patient with osteomyelitis.

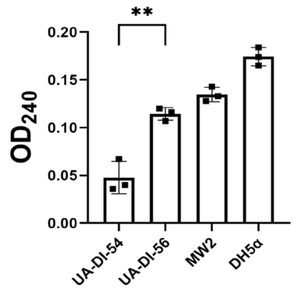
We have discovered a frameshift mutation in *eno* and potentially a novel mechanism in *S. aureus* in the switch to SCV

INCREASED BIOFILM FORMATION



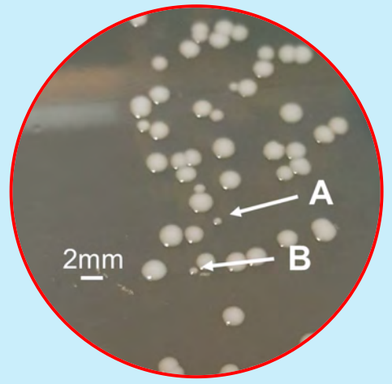
- Real-time biofilm formation was measured using xCELLigence
- Biofilm formation was decreased in the sSCV UA-DI-54

DECREASED ENOLASE ACTIVITY



- Enolase catalyses the conversion of 2-PG to PEP, the penultimate step in glycolysis.
- Enolase activity was measured by production of PEP at a wavelength of 240nm
- The sSCV UA-DI-54 has deficient enolase activity

** = p-value < 0.005



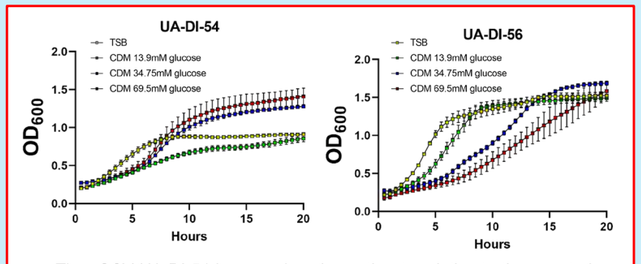
UA-DI-54 - sSCV

A: Retains SCV phenotype after subculture

UA-DI-56 - nsSCV

B: Reverts to the normal cell type

ATYPICAL GROWTH RATE WITH GLUCOSE



- The sSCV UA-DI-54 has a reduced growth rate relative to the reverted SCV UA-DI-56
- Increased glucose is typically detrimental to *S. aureus* growth, displayed in the reverted SCV UA-DI-56
- The sSCV UA-DI-54 growth rate increase proportionally with glucose concentration

CONCLUSIONS

The sSCV phenotype was associated with:

- Frameshift mutation in *eno* and reduced enolase function
- Reduced growth rate proportional to glucose concentration and decreased rate of biofilm formation

What is next?

- Characterisation of the metabolic pathways that underly the changes in growth patterns in the enolase deficient sSCV
- Cell culture based models of infection to determine changes in adhesion, invasion and cytotoxicity

References:

Lee J., Mashayamombe M., Walsh T.P., Kuang B.K.P., Pena G.N., Vreugde S., Cooksley C., Carda-Diéguez M., Mira A., Jesudason D., Fitridge R., Zilm P.S., Dawson J., Kidd S.P. (2023), "The bacteriology of diabetic foot ulcers and infections and incidence of Staphylococcus aureus Small Colony Variants" *J Med Microbiol*

Healthcare-associated *Staphylococcus aureus* bloodstream infections associated with peripheral intravenous cannulae in Western Australian hospitals, 2019 to 2023

Khui Hung (Claire) Lee[1], Liana Varrone[1], Inutu Kashina[1], Olivia Kamau[1], Lisa Nicolaou[1], Melanie Trainor[1], Rebecca Hogan[1], Rebecca McCann[1]

[1]Infection Prevention, Policy and Surveillance Unit, Communicable Disease Control Directorate, Western Australian Department of Health

BACKGROUND

Reporting of healthcare-associated *Staphylococcus aureus* bloodstream infections (HA-SABSIs) via the Healthcare Infection Surveillance Western Australia (HISWA) program is overseen by the Infection Prevention Policy Surveillance Unit (IPPSU). It has been mandatory since 2016 as described in the *Insertion and Management of Peripheral Intravenous Cannulae in Healthcare Facilities Policy*.[1] The publication of this mandatory policy was prompted by the increasing rates of HA-SABSIs attributable to intravascular devices (IVDs) in WA hospitals. Historical data indicated that the majority of HA-SABSIs were attributable to IVDs. Modifications were made to the HISWA database in 2019 to allow for collection of data on the type of IVD, and the dwell time for peripheral intravenous cannula (PIVC).

OBJECTIVE

To examine surveillance data on HA-SABSIs in WA between 2019 and 2023, focusing on those infections associated with PIVC to assess the impact of the 2016 *Insertion and Management of Peripheral Intravenous Cannulae in Healthcare Facilities Policy* on infection rates.

METHODOLOGY

Data on HA-SABSI reported to HISWA between 2019 and 2023 were analysed using RStudio.[2] HA-SABSI events were categorised by attributable source, with further stratification by IVD type. PIVC-related HA-SABSI were further categorised by the documented duration that the PIVC was in situ.

RESULTS

Completeness of attributable source data recorded within HISWA increased from 85% in 2019 to 100% by 2020 – a level maintained thereafter. The total HA-SABSI rate was 0.61 per 10,000 bed days in 2019 compared with 0.67 in 2023 (Figure 1). The attributable source for the majority of HA-SABSI are IVDs and this is across all years (Figure 2). Of IVD-related HA-SABSIs, the proportion attributed to PIVCs increased from 42% in 2019 to 65% in 2023 (Figure 3). Stratification by dwell time for PIVC-related HA-SABSIs identified 132 infections (51%) with a PIVC in situ for 72 hours and less, and 40 (16%) in situ for more than 72 hours (Figure 4). The remaining 85 (33%) PIVC-related HA-SABSI had inadequate insertion documentation to identify the time in situ.

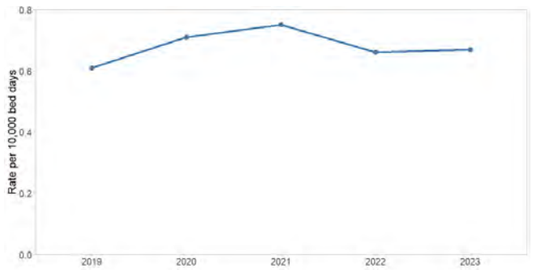


Figure 1: Total healthcare-associated Staphylococcus aureus bloodstream infection rates, 2019 to 2023.

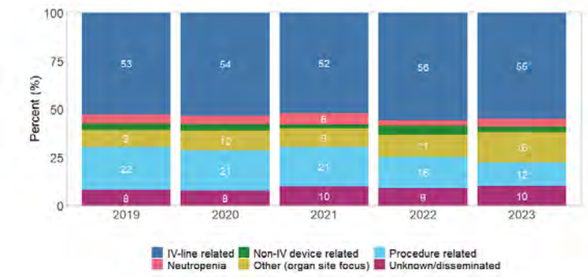


Figure 2: Percentage of HA-SABSI by attributable source, 2019 to 2023.

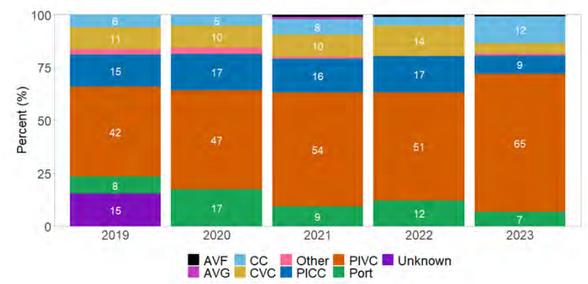


Figure 3: Percentage of HA-SABSI by intravascular device type, 2019 to 2023.

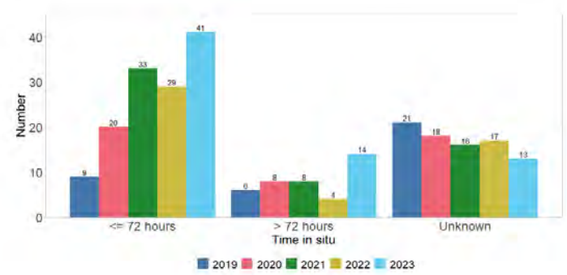


Figure 4: PIVC by time in situ, 2019 to 2023.

CONCLUSION

Despite current infection prevention strategies that are in place in WA hospitals based on the 2016 PIVC Policy, the incidence and the rate of HA-SABSIs in WA attributed to intravascular devices continue to increase. Amendments made to the HISWA database have increased the completeness of data capture, however compliance with documentation requirements by hospital staff is still not optimal to allow for thorough analysis of PIVC dwell times. WA's surveillance system has repeatedly identified use of PIVCs as a major risk factor for HA-SABSIs, and further interventions are required to address this beyond mandatory policy development and review.

CONTACT

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[1]Insertion and Management of Peripheral Intravenous Cannulae in Healthcare Facilities Policy. (2016). Retrieved from https://www.health.wa.gov.au/~/media/Files/Corporate/Policy-Frameworks/Public-Health/Policy/Insertion-and-Management-of-Peripheral-Intravenous-Cannulae/MP38-Insertion-and-Management-of-Peripheral-Intravenous-Cannulae.pdf.

[2]R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2020.

Government of Western Australia Department of Health

The Diagnostic Utility of Imaging Modalities for Paediatric *Staphyloccocus aureus* Bacteraemia (SAB)

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BACKGROUND

Staphylococcus aureus bacteraemia (SAB) is an invasive infection most commonly causing osteoarticular infection in the paediatric population. (1) Other common foci of SAB infections include pulmonary infection (1) and infective endocarditis with high mortality. (2)

Diagnosis of these foci relies on a combination of clinical assessment and diagnostic imaging. Different imaging modalities are used for paediatric SAB with variable sensitivity and specificity depending on the organ system involved and patient related variables. There are limited evidence-based recommendations guiding an approach to imaging to determine the body system affected in children with SAB.

AIM AND OBJECTIVES

Aim: To evaluate **clinical and microbiological variables** affecting the **diagnostic yield** of imaging performed for paediatric **SAB** with different foci of infection.

Primary Objective :

- Descriptive analysis of clinical variables, microbiological variables and imaging performed.
- Diagnostic yield of imaging performed to inform the likely prevalence in a cohort of children with SAB.

Secondary Objective:

- Explore the patient and microbiological variables associated with osteoarticular, pleuropulmonary and endovascular infection in children with SAB.
- Healthcare costs of imaging performed.

METHODS

Data including diagnostic modalities were examined from a prospective multicentre cross-sectional study involving children ≤18 years of age with SAB hospitalised over a 2-year period (2017-2018) in Australia and New Zealand. (3) Descriptive analysis for clinical, microbiological variables and imaging performed were conducted. Imaging cost was obtained according to the 2017 Independent Health and Aged Care Pricing Authority (IHACP) index costings.

$$\text{Diagnostic yield} = \frac{\text{number of children with a positive diagnostic image (focus of infection) by anatomical site (e.g. pneumonia)}}{\textbf{total number} \text{ of children } \textbf{with imaging performed by anatomical site (e.g. pleuropulmonary)}}$$

Patient/microbiological variables → Chi square/Fisher's exact test/Mann-Whitney U test → Binary regression analysis

RESULTS

Osteoarticular infection

- Diagnostic yield = **68% for osteomyelitis** with X-ray being the most frequent imaging modality used
- Age 12-18 years old (odds ratio [OR] 5.53 [95% confidence interval [CI], 1.49 - 20.81]) and multifocal infection (OR 4.17 [95% CI, 1.39 - 12.53]) were associated with a higher likelihood of an osteoarticular focus of infection.

Pleuropulmonary infection

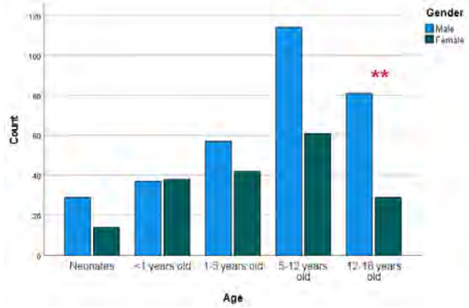
- Diagnostic yield = **21% for pneumonia** with X-ray being the most employed imaging modality
- Multifocal infection (OR 11.80 [95% CI, 2.17 to 64.30]) and ICU admission (OR 4.66 [95% CI, 1.84 to 11.81]) were associated with an increased likelihood of pleuropulmonary infection.

Endovascular infection

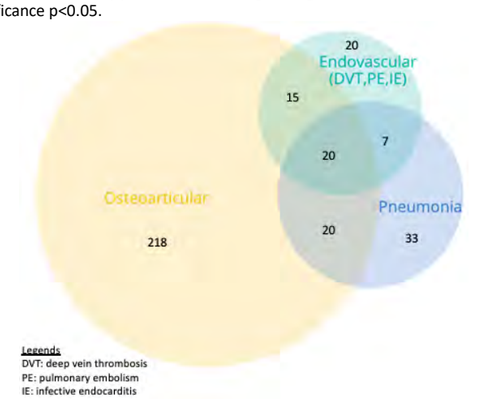
- Diagnostic yield = **12% for endocarditis** with transthoracic echocardiogram (TTE) being the most employed imaging modality
- Patients with multifocal infection (OR 44.314 [95% CI, 5.592 to 351.138]) and congenital heart disease (CHD) (OR 59.078 [95% CI, 12.603 to 276.938]) had an increased probability of endocarditis.

CONCLUSION

- **Osteoarticular imaging** had the highest diagnostic yield confirming osteomyelitis .
  - **X-ray** (n=261) being the most frequently performed radiological test while **MRI** (n=217) being more sensitive has the highest cost associated.
- Specific variables such as CHD increased the probability of endocarditis.
- This study provides important insights into the diagnostic imaging performed and cost associated with paediatric SAB.
- It also shows how different variables can increase the likelihood of a foci of infection and inform those most likely to benefit from diagnostic imaging.

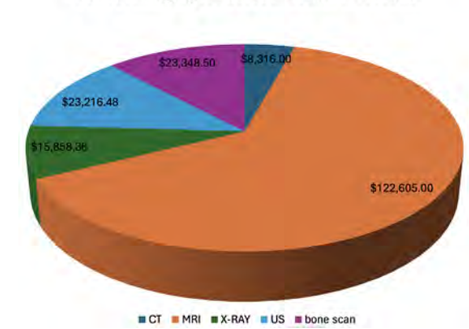


Graph 1: Proportion of children who had imaging performed to examine for a SAB infective focus by patient variables: age and gender. **statistical significance p<0.05.



Venn diagram 1: Total number of participants (n=333) with a positive infective foci for S. aureus bacteraemia on diagnostic imaging for osteoarticular (osteomyelitis and septic arthritis), endovascular (including infective endocarditis, deep vein thrombosis and/or pulmonary embolism) and or pleuropulmonary foci of infection.

Cost of imaging for osteoarticular infectons



Pie chart 1: The total costs of imaging for suspected osteoarticular infection in children with SAB was AUD$193,344.34.

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Can commensal *Staphylococcus felis* kill methicillin-resistant *Staphylococcus pseudintermedius in-vitro*?

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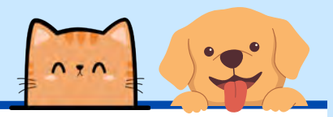
1. Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, NSW, Australia
2. Sydney Infectious Diseases Institute, The University of Sydney, NSW, Australia



1
BACKGROUND:

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), a major pathogen of dogs that primarily affects the skin, is an occasionaly zoonotic pathogen that is becoming increasing prevalent. There is a pressing need to develop novel therapeutics to sustain treatment capabilities.

Antimicrobial peptides (AMPs) derived from commensal organisms offer a potential solution as they can disrupt cellular integrity of pathogenic bacteria.

A single strain (C4) of the coagulanase-negative commensal of cats, *Staphylococcus felis,* has been shown to produce AMPs that successfully inhibit MRSP multi-locus sequence type (ST)71 both *in-vitro* and *in-vivo* (O'Neill et al., 2021). Although promising, it is unknown whether *S. felis* C4 AMPs are effective against a wider range of MRSP STs.

2
AIM:

To investigate the inhibitory effects of *Staphylococcus felis* AMPs on multiple STs of MRSP using an in-vitro model.

3
METHODS:

Thirty clinical isolates of MRSP from Australian Veterinary Diagnostic Laboratories were subcultured onto sheep blood agar plates at 37°C.

Staphylococcus felis C4, previously isolated from the skin of a healthy cat, was grown in tryptose soy broth (TSB) and filtered with a 2um filter to produce a cell-free supernatant. The supernatant was diluted to a 12.5% solution in TSB.

One colony of each MRSP isolate was inoculated into 150µl of 12.5% cell-free *S. felis* supernatant in a 96-well plate, in triplicate. In the control condition, triplicates of each MRSP isolate were similarly inoculated into 150µl of TSB. Plates were incubated overnight at 37°C (Figure 1).

Optical densities were measured, and efficacy was assessed via a linear mixed model incorporating random effects for replicate variability, with statistical significance set at $p < 0.05$.

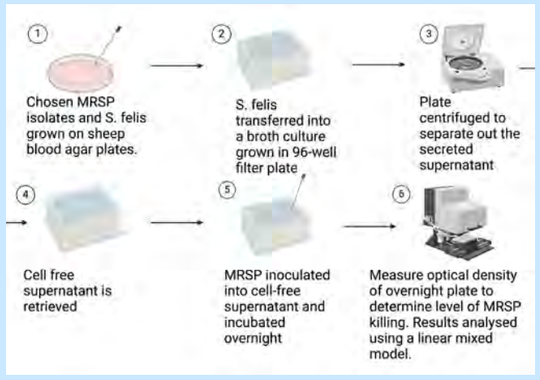


Figure 1: Protocol used to test *S. felis* against MRSP.

4
RESULTS

Significant suppression of MRSP growth across all sequence types was observed in the presence of *S. felis* C4 supernatant compared to the TSB control ($p<0.001$; Figure 2).

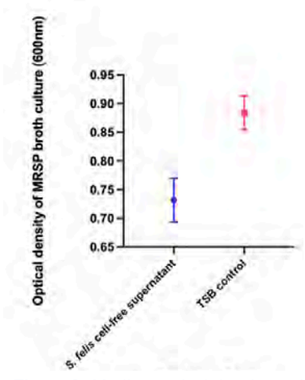


Figure 2: Median optical density and 95% confidence intervals of 30 MRSP isolates grown in *S. felis* cell-free supernatant compared to growth in a TSB control.

5
DISCUSSION

- *Staphylococcus felis* supernatant significantly reduced the growth of multiple STs of MRSP, even at the relatively low concentration of 12.5%.
- *Staphylococcus felis* C4 and its AMPs offer a promising solution to the issue of antimicrobial resistance in *S. pseudintermedius.*
- These findings pave the way to a potentially novel treatment modality involving topical probiotics as therapy for MRSP skin infections in dogs. Further research in a canine model needs to be done to investigate the real world efficacy of *S. felis* AMPs against MRSP.

O'Neill, A. M., Worthing, K. A., Kulkarni, N., Li, F., Nakatsuji, T., McGrosso, D., Mills, R. H., Kalla, G., Cheng, J. Y., Norris, J. M., Pogliano, K., Pogliano, J., Gonzalez, D. J., & Gallo, R. L. (2021, 2021/10/19). Antimicrobials from a feline commensal bacterium inhibit skin infection by drug-resistant S. pseudintermedius. eLife, 10, e66793. https://doi.org/10.7554/eLife.66793

PknB kinase directly controls the WalKR system and modulates cell wall antibiotic resistance in *Staphylococcus aureus*

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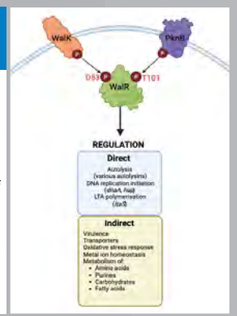
[2]Department of Microbiology and Immunology, The Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia.
[3]Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

For further information contact **msuppiperez@student.unimelb.edu.au**

Background

- Antimicrobial resistance (AMR) continues to be a threat health and supposes an economic burden worldwide. Examples are Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-intermediate *S. aureus* (VISA), or Vancomycin-resistant *S. aureus* (VRSA).
- Mutations in *S. aureus* two-component regulatory systems (***walKR***, *vraRS* and *graRS),* genes associated with cell wall synthesis or hydrolysis, ***sle1*** and *msrR*, post-translational modification, ***stp1*** and *clpP,* have been linked with the VISA phenotype.
- WalKR system is the only **essential** two-component system (TCS) in *S. aureus.* It regulates genes involved in cell wall homeostasis.
- We have described a point mutant, ***walR1***, that has a VISA-like phenotype. The *walR1* mutation alters the proposed phosphorylation site of Ser/Thr kinase, **PknB (T101M)**.
- *walR1* presents low-level resistance to vancomycin and extreme sensitivity to **tunicamycin**.
- Here, we have explored the role of the T101 residue in phosphorylation by PknB, and how this might impact WalKR signalling in *S. aureus* ATCC29213.
- Identifying "weak spots" in multidrug-resistant pathogens might facilitate the discovery of new drugs that could target those specifically.



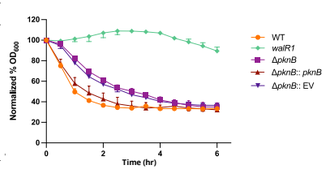
Results

1) Phenotypic comparison between *walR1* and Δ*pknB* mutants

A

	Wild type	*walR1*	Δ*pknB*	Δ*pknB* :: *pknB*	Δ*pknB* :: EV
Tunicamycin	8	0.125	1	8	1
Vancomycin	1	2	1	2	2
Oxacillin	0.25	0.125	0.125	0.125	0.125
Fosfomycin	16	16	16	32	16
Ciprofloxacin	0.5	0.25	0.5	0.5	0.5
Rifampicin	0.00195	0.0156	0.00195	0.0039	0.0039
Kanamycin	8	8	8	8	8

B



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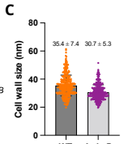

D

Phase	Strain	Non-dividing	Dividing
Logarithmic	Wildtype	44.9%	55.1%
	Δ*pknB*	43.1%	56.9%
Stationary	Wildtype	70.8%	29.2%
	Δ*pknB*	72.9%	27.1%

E

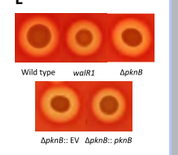

Fig 1. A) Minimum inhibitory concentration for a panel of antibiotics of Wild type (WT), *walR1* and Δ*pknB* strains (as well as complemented strains). **B)** *In vivo* autolysis assay of the previous strains. Results are from 3 independent biological replicates. Median and error range. **C)** Cell wall size of WT and Δ*pknB* strains assessed by measuring over 350 cells. ± SD. **D)** Percentage of cells in either non-dividing or dividing stated based on the 350 cells from transmission electron microscopy. **E)** Strains plated in sheep blood agar (SBA) to determine haemolysis.

2) Δ*pknB* mutant influences global gene expression and the direct WalKR regulon

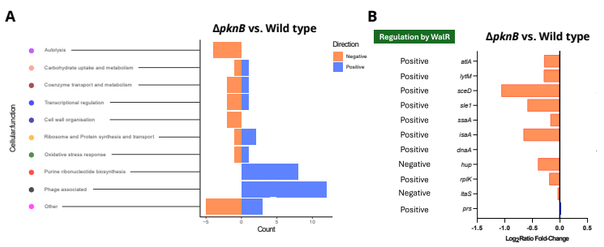

Fig 2. A) The number of genes in each category that is positively or negatively regulated (FDR < 0.05, log2 Fold-Change <-0.5 or >0.5). Category "other" includes hypothetical genes or proteins with unknown function. **B)** Impact of Δ*pknB* mutation on the expression of the direct WalKR regulon compared to wildtype cells.

3) PknB interacts with WalR and other response regulators involved in cell wall metabolism *in vivo*

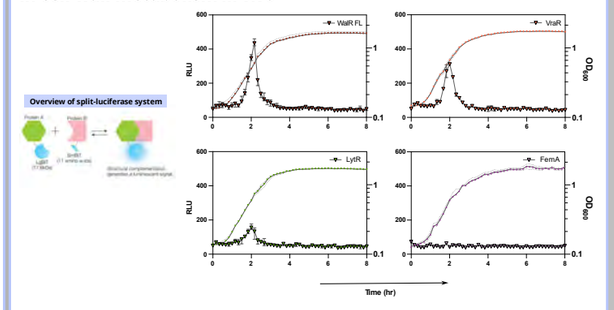

Fig 3. Kinetic interactions of PknB with various proteins in *S. aureus*. Analysis of the interaction of PknB-LgBIT with various proteins fused to SmBIT (split-luciferase system) throughout growth in ATCC29213. In order: full-length WalR, VraR, LytR and FemA. Results are representative of three repeat experiments.

4) PknB phosphorylation promotes WalR dimerisation through key T101 residue

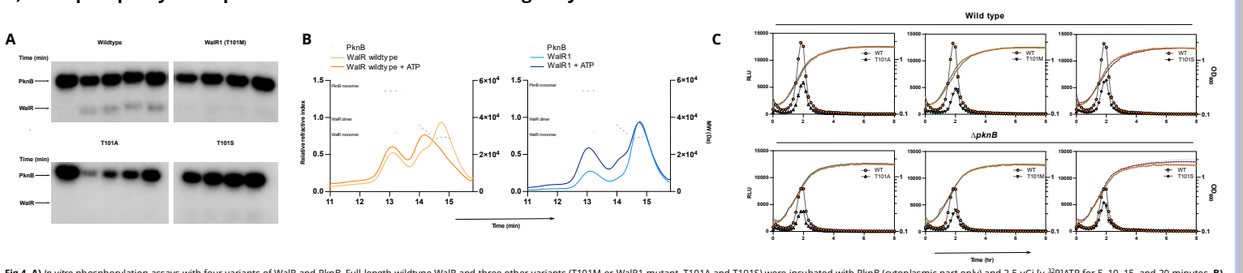

Fig 4. A) *In vitro* phosphorylation assays with four variants of WalR and PknB. Full-length wildtype WalR and three other variants (T101M or WalR1 mutant, T101A and T101S) were incubated with PknB (cytoplasmic part only) and 2.5 uCi [γ-^{32}P]ATP for 5, 10, 15, and 20 minutes. **B)** Elution profiles of wildtype (in orange) and WalR1 (T101M; in blue) after phosphorylation by PknB and analysed by SEC-MALS. Molecular weight (Da) was calculated using ASTRA software. Results are the median from three independent experiments. **C)** Kinetic interactions between different T101 mutants to assess *in vivo* dimerisation in *S. aureus* (wildtype and Δ*pknB* background) . Results are representative of three repeat experiments.

Conclusions

1. Δ*pknB* mutation confers **increased sensitivity** to tunicamycin and oxacillin. It shows a **modest** defect in autolysis, but no change in cell wall thickness.
2. *walR1* and Δ*pknB* mutations are mechanistically **distinct**, with *walR*^{T101M} mutation presenting a more severe phenotype.
3. T101M mutation averts PknB phosphorylation.
4. T101 residue influences **dimer** formation of WalR, and T101 phosphorylation is important for efficient dimerisation.

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- Image Split-luciferase system: https://www.promega.com/~/media/images/resources/figures/13400-13499/13423ma.jpg

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Breakpoint NEWS

Diversity of MRSA Clones Reported in Western Australia (WA)

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2. Antimicrobial Resistance and Infectious Diseases Research Laboratory, Murdoch University, WA

Introduction

In Western Australia (WA) Methicillin-Resistant Staphylococcus aureus (MRSA) is a notifiable organism as per mandatory policy outlined by Department of Health, WA. This policy ensures early detection, isolation and appropriate therapy where required and includes a comprehensive and effective outbreak, identification, and management policy. The Western Australian public and private medical laboratories refer all MRSA isolates to the PathWest Gram-Positive Typing Laboratory (GPTL) for characterisation. All MRSA isolates are electronically tagged with micro-alert status B or C with micro-alert B MRSA isolates unlikely to spread in the healthcare environment and micro-alert C MRSA isolates linked with increased virulence or transmissibility and potential to spread in the healthcare environment.

Methods

All MRSA are characterised by phenotypic and genotypic methods. Phenotypic method includes urease production test and antibiogram susceptibility testing by disc diffusion (CLSI) to gentamicin, erythromycin, tetracycline, ciprofloxacin, trimethoprim, fusidic acid and rifampicin. The genotypic methods used are Real-Time PCR targeting *mecA*, *nuc*, Panton-Valentine Leukocidin (PVL) and *aroE*; coagulase gene PCR-restriction fragment length polymorphism (RFLP); Pulsed-Field Gel Electrophoresis (PFGE), DNA microarray and whole genome sequencing. The MRSA isolate is determined to be either a micro-alert B or C. Clone type is reported for PVL-positive and/or micro-alert C MRSA. Non-micro alert C PVL-negative MRSA are reported as “Micro-alert B, PVL negative MRSA”.

Results

- 8,473 non-duplicate MRSA isolates were characterised from 01 July 2022 to 30 June 2023.
- 573 (6.8%) isolates were characterised as Micro-alert C PVL-negative Healthcare-associated MRSA infections (HA-MRSA) (Table 1).
- The dominant HA-MRSA clone was PVL negative UK15/EMRSA-15 (ST22-IV [2B]; n=562; 98%) followed by Aus-2/3 EMRSA (ST239-III [3A]; n=5; 0.9%), New York Japan MRSA (ST5/ST764-II [2A], n=2, 0.3%), UK 16 (ST36-II [2A]; n=2; 0.3%) and ST225-II [2A] (n=2; 0.3%) (Table 2).
- 302 (3.6%) isolates were characterised as Micro-alert C Community-associated MRSA (CA-MRSA). Three CA-MRSA clones reported to cause outbreaks were identified : PVL-positive ST22-IV [2B] (n=184, 61%), USA300 (ST8-IV [2B]; n=102; 34%) and Bengal Bay MRSA (ST772-V [5C2]; n=16; 5.2%)
- 7,598 (90%) isolates were identified as Micro-alert B CA-MRSA.
- Of the 7,598 isolates, 3,643 (43%) were Micro-alert B PVL negative CA-MRSA and 3,955 (47%) were Micro-alert B PVL positive CA-MRSA.
- Of the 3,955 Micro-alert B PVL positive CA-MRSA, the predominant clones were Queensland Clone (ST93-IV [2B]; n=2,295; 58%) and WA MRSA 121 (ST5-IV [2B]; n=1,144; 29%) (Figure 1).
- A downward trend (X^2 2,147, P<0.01) in the proportion of HA-MRSA from 22% in 2003/2004 to 6.8% in 2022/2023 (Figure 2).
- An upward trend (X^2 2,147, P<0.01) in the proportion of CA-MRSA from 78% in 2003/2004 to 93% in 2022/2023 (Figure 2).

Conclusions

Since 1991, CA-MRSA clones have been associated with a significant increase in MRSA notifications in WA. Despite having a high prevalence in the community, CA-MRSA have not become endemic in WA hospitals. In addition, the successful mandatory policy implemented by the Department of Health has also prevented HA-MRSA from becoming established in WA hospitals. This highlights the importance of a central epidemiological typing laboratory which uses rapid molecular genotyping to distinguish clones that can become endemic in the healthcare setting. Furthermore, the surveillance data combined with the genomic data can provide enhanced information on the emergence, transmission, and the evolution of MRSA clones in WA.

Table 1. MRSA isolates by Micro-alert status in WA, July 2022 to June 2023

Micro-alert MRSA	Total	Proportion (%)
Micro-alert C, PVL negative	573	6.8
Micro-alert C, PVL positive	302	3.6
Micro-alert B, PVL negative	3,643	43
Micro-alert B, PVL positive	3,955	47
Total	**8,473**	**100**

Table 2. Micro-alert C HA-MRSA in WA, July 2022 to June 2023

MLST-SCC*mec*	Clone	Total	Proportion (%)
ST22-IV [2B]	UK 15/EMRSA-15	562	98
ST239-III [3A]	Aus-2/3 EMRSA	5	0.9
ST5-II [2A]	New York Japan MRSA/USA100	2	0.3
ST36-II [2A]	UK 16	2	0.3
ST225-MRSA-II [2A]		2	0.3
Total HA-MRSA		**573**	**100**

Table 3. Micro-alert C CA-MRSA in WA, July 2022 to June 2023

MLST-SCC*mec*	Clone	Total	Proportion (%)
ST22-IV [2B]	PVL-positive ST22	184	61
ST8-IV [2B]	USA300	102	34
ST772-V [5C2]	Bengal Bay MRSA	16	5.2
Total CA-MRSA		**302**	**100**

Figure 1: Micro-alert B PVL positive MRSA in 2022/2023

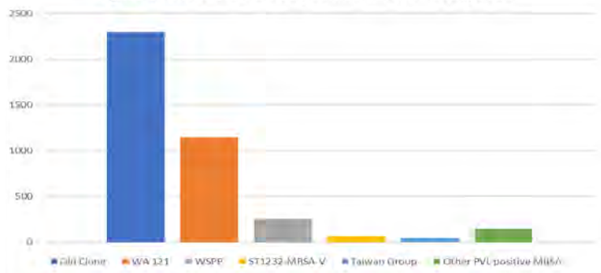
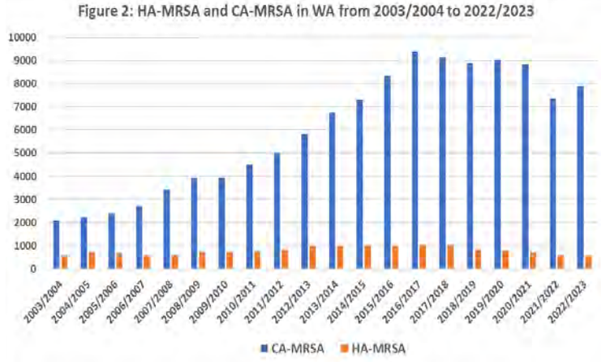


Figure 2: HA-MRSA and CA-MRSA in WA from 2003/2004 to 2022/2023



Acknowledgements
We would like to gratefully acknowledge the scientific and technical staff of Gram-Positive typing Laboratory and Department of Microbiology (PathWest Fiona Stanley hospital), Antimicrobial Resistance and Infectious Diseases (AMRID) Research Laboratory, College of Science, Health, Engineering and Education of Murdoch University, Department of Health, Western Australia, all public and private medical microbiology laboratories for referring MRSA isolates (Australian Clinical Laboratories, Clinipath, PathWest Laboratory Medicine WA and Western Diagnostic Laboratories).

FSHMA202 4929/2005

Genomic Epidemiology of Clonal Complex 1 *Staphylococcus aureus* from Remote Communities in Western Australia

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[2]Department of Microbiology, PathWest Laboratory Medicine-WA, Fiona Stanley Hospital, Murdoch, Australia
[3]Sequencing and Bioinformatics, Bacteria, Parasites & Fungi, Statens Serum Institut, Copenhagen, Denmark

Introduction

- In the 1990s, community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first identified in remote communities in Western Australia (WA).
- ST1-MRSA-IV (colloquially known as WA-1 CA-MRSA) from the clonal complex 1 (CC1) lineage persistently dominated in WA over the next three decades.
- Although little is known about the genomic diversity of WA-1 CA-MRSA in WA, it is believed its success is due to the carriage of few antimicrobial resistance (AMR) and virulence genes.

Aim: Investigate the genomic epidemiology of CC1 *S. aureus* isolated in WA from 1995 to 2003.

Methods

- In a study by O’Brien *et al., S. aureus* isolates from healthy carriers residing in remote WA communities from 1995 to 2003 were characterised using pulsed-field gel electrophoresis.
- Short-read sequencing was performed on CC1 *S. aureus* isolates identified by O’Brien *et al.*.
- The SCC*mec*Finder tool was used to identify SCC*mec* types.
- The genomes were screened for virulence determinants using a BLAST interface.
- NCBI AMRFinderPlus tool was used to screen for antimicrobial resistance (AMR) genotypic markers.
- The phylogenetic tree was constructed with a maximum-likelihood algorithm using a core-genome SNP alignment.
- The Bayesian Coalescent method was used to infer the expansion of the CC1 lineage.

Results

- Of the 137 CC1 *S. aureus* isolates characterised by O’Brien *et al.*, **86.1%** (n=**118**) were viable and **successfully analysed**.
- **Three STs** were identified: **ST1** (n=96), **ST762** (n=21), and **ST761** (n=1).
- **Three clades** were identified: clade **A** (n=22), clade **B** (n=9), and clade **C** (n=86) (Figure 1).

In clade **B**, **98** non-synonymous clade-specific **SNPs** were identified, and **39** non-synonymous clade-specific **SNPs** were identified in clade **C**.

- All CC1 isolates were *agr* type III/capsular serotype 8.
- **Seven** (5.9%) CC1 **isolates** were **PVL-positive**, and **IEC genes** were detected in **114** (96.7%) **isolates** (Figure 1).
- **All** CC1 **MRSA** isolates harboured **SCC*mec* IVa [2B]**. Genomic regions within the **SCC*mec* IVa [2B]** elements were **identical** in **most** CC1 MRSA isolates (Figure 1).
- **MRSA** isolates in **Clade B** and **C** (highlighted in red) expanded in **1995** (95% highest posterior density [HPD] 1993 – 1997) and **1984** (95% HPD 1982 – 1986), respectively (Figure 1).
- **Genotypic markers** conferring resistance to **β-lactams**, **chloramphenicol**, **erythromycin**, **fluoroquinolones**, and **tetracycline** were identified (Figure 1).
- **Simultaneous movement** of isolates between communities, as inferred by the phylogeographic map, occurred from **1992** to **2000** (Figure 2).

Conclusions

- Three phylogenetically distinct clades were identified in the WA CC1 *S. aureus* lineage.
- The fitness of the CC1 lineage can be attributed to the carriage of a few AMR and virulence genetic determinants.
- The simultaneous movement of CC1 *S. aureus* isolates suggests the movement of Aboriginal inhabitants across the state.

Figure 1. Maximum-likelihood SNP phylogeny of 118 CC1 *S. aureus* isolates isolated from WA communities from 1995 to 2003.

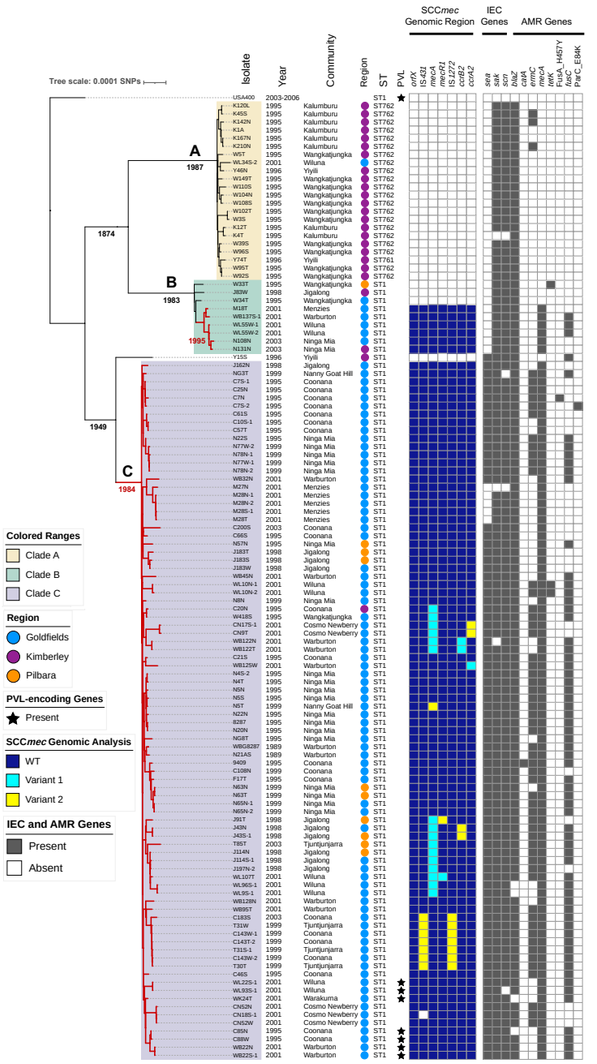
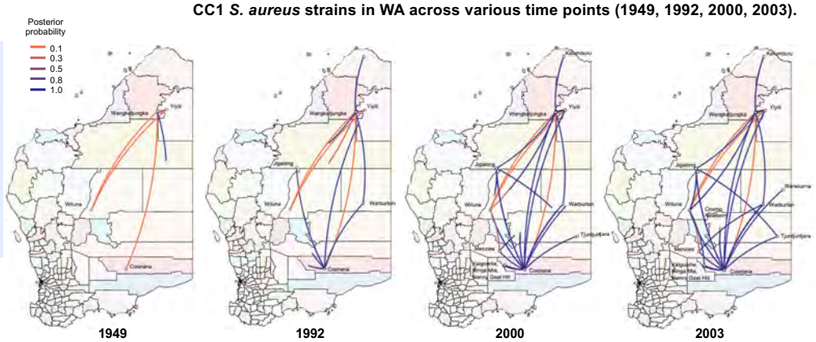


Figure 2. A phylogeographic posterior probability map of the distribution of CC1 *S. aureus* strains in WA across various time points (1949, 1992, 2000, 2003).



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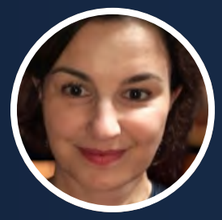

Deirdre Pinto
Chronic UTI Australia

Monash-affiliated Speakers:

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Friday 15th November 2024 – The Alfred Innovation & Education Hub, 75 Commercial Road, Melbourne		
8:45 – 9:00	Welcome and introduction	**Dr Malcolm Starkey**
Session 1	**Urinary tract infection - patient voices and plenary**	**Chair: Dr Iain Abbott**
9:00 – 9:30	• **Deirdre Pinto (Chronic UTI Australia)** – *Hearing patient voices: capturing the impacts of chronic UTI*	
9:30 – 10:30	• **Prof Jennifer Rohn (University College London)** – *Surface tension: using a human 3D urothelial microtissue model to understand infection dynamics and treatment resistance*	
10:30 - 11:00	Morning tea & poster viewing	
Session 2	**Urinary tract infection, microbiome & ECR talks**	**Chair: Prof. Catriona Bradshaw**
11:00 – 11:40	• **Prof Kirsty Buising (Royal Melbourne Hospital)** – *Antibiotics for urinary tract infections*	
11:40 – 12:10	• **Dr Iain Abbott (Alfred Health)** - *Mimicking human bladder function & UTI treatment dynamics*	
12:10 – 12:40	• **Dr Erica Plummer (Melbourne Sexual Health Centre)** - *Novel infectious causes of male urethritis*	
12:40 – 13:00	• **Rapid fire early career researcher talks** – *selected from abstract submissions (2x 10 mins)*	
13:00 - 14:00	Lunch break & interactive poster session	
Session 3	**Chronic kidney disease, transplantation and organoids**	**Chair: A/Prof. Melinda Coughlan**
14:00 – 14:30	• **Kidney Health Australia** – *The impact of kidney disease in Australia*	
14:30 – 15:10	• **Prof Merlin Thomas (Monash University)** - *RNA therapy for the treatment of CKD*	
15:10 – 15:40	• **Prof. Michaela Lucas (Uni Western Australia)** – *T cells in kidney transplantation*	
15:40 – 16:10	• **Dr Alex Combes (Monash BDI)** - *Modelling hypoxic injury & repair in human kidney organoids*	
16:10 – 16:45	Afternoon tea & poster viewing	
Session 4	**Rare kidney diseases**	**Chair: Dr Nirmal Bhatt**
16:45 – 17:30	• **Prof. Michael Hickey (Monash CID) -** *Monocyte subset trafficking in acute glomerulonephritis*	
17:30 – 18:10	• **Dr Kim O'Sullivan (CID)** - *Targeting inflammatory extracellular DNA in autoimmune kidney disease*	
18:10 – 18:20	Closing remarks	**Dr Malcolm Starkey**
19:00 – 22:00	Symposium dinner and social networking function	**The Commons Collective**

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