~~Extended-spectrum β-lactamases (ESBLs): Analysis of prevalence in residential aged care facilities (RACFs) compared with the community in local Sydney~~Penicillin treatment of PSSA-B: defining clinical outcomes by definitive treatment with penicillin vs flucloxacillin; and penicillin disc diffusion zone size and the presence of *blaZ*

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Protocol Version - 1

Protocol Date: 29/04/2022

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC *National Statement on Ethical Conduct in Human Research (2007)* and the *Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95)* and consistent with the principles that have their origin in the Declaration of Helsinki. Compliance with these standards provides assurance that the rights, safety and well-being of study participants are respected.

1. BACKGROUND AND INTRODUCTION

1.1. Disease/proposed study background

S. aureus bacteraemia is associated with a high mortality, 15-25% at 90 days. There has been an increase in the percentage of penicillin-susceptible *S. aureus* bacteraemia (PSSA-B) in recent years.

1.2. Research question

1. For patients in the ASSOP cohort with PSSA-B, is benzylpenicillin treatment non-inferior to flucloxacillin with respect to 30-day mortality?

2. For patients with PSSA-B as determined by the clinical laboratory, is there an association between penicillin disc diffusion zone size, or the presence of *blaZ,* and mortality, in patients treated with penicillin for PSSA-B.

1.3. Rationale for performing the study

S. aureus is frequently (~85% of isolates) resistant to penicillin due to production of a penicillinase encoded by *blaZ.* Phenotypic detection of penicillinase production by the CLSI method, using a 10mcg penicillin disk (P10) has a reported sensitivity of 64-89% and by the EUCAST method, using a 1mcg penicillin disc (P1) a 90-100% sensitivity rate, when measured against molecular detection of *blaZ*. The lack of complete detection of all isolates with *blaZ* has led to some hesitation in using benzylpenicillin as definitive treatment. Methods which are no longer recommended include colorimetric cefinase detection, with lower sensitivity. This data suggests a very major error rate (calling an isolate susceptible when it is genotypically resistant) of 10-35% for the CLSI method and 0-10% for the EUCAST method. Given approximately 10% of isolates with automated broth microdilution MIC $\leq$0.12 mg/L have *blaZ,* this would translate to 0.5-2% of possible PSSA being miscalled as susceptible to penicillin.

Benzylpenicillin has theoretical pharmacokinetic advantages over flucloxacillin, and the safety of flucloxacillin has been questioned. Observational data suggests that PSSA-B patients treated with benzylpenicillin have lower mortality and fewer treatment-related complications.

This observational data, based on penicillinase detection with cefinase and the P1/P10 disc methods, still shows lower mortality for patients treated with benzylpenicillin. The third cohort study used *blaZ* molecular detection for isolates with a P1 zone size of $\geq$26mm. However, it raises the question of whether the presence of *blaZ* has a clinical impact, given that it is likely that the presence of *blaZ* would have been missed in 0.5-2% of these patients. It is possible that the presence of *blaZ* may not affect treatment success for isolates with a larger P1 zone diameter.

Study Title	~~Extended-spectrum β-lactamases (ESBLs): Analysis of prevalence in residential aged care facilities (RACFs) compared with the community in local Sydney~~Penicillin treatment of PSSA-B: defining clinical outcomes by penicillin disc diffusion zone size and the presence of blaZ
Objectives	The null hypothesis is that benzylpenicillin treatment is inferior to flucloxacillin treatment for PSSA-B as determined by phenotypic testing in the clinical microbiology laboratory. The secondary null hypothesis is that there is no association between penicillin disc diffusion zone size, or the presence of *blaZ,* and mortality, in patients treated with penicillin for PSSA-B. Primary: 1. To examine the association between treatment with benzylpenicillin vs flucloxacillin for patients with penicillin-susceptible *S. aureus* bacteraemia (PSSA-B) and 30-day mortality in a cohort of patients from Australian hospitals 2013-21 (ASSOP, data collected by institutions participating in AGAR) 2. For those patients treated with benzylpenicillin, and isolates available, to examine mortality rate by penicillin disc zone size, and the presence or absence of *blaZ* on molecular testing.
Study Design	1) Retrospective Observational Analytical Study. 2) Laboratory study of penicillin susceptibility of PSSA isolates
Planned Sample Size	Approximately 400 patients per calendar year.
Selection Criteria	Inclusion • ASSOP patient's *S. aureus* blood culture isolate was categorized as PSSA by the treating institution's clinical laboratory • Patient was treated with either benzylpenicillin or flucloxacillin as deemed by the institution's AGAR investigator. • Secondary outcome: patients treated with benzylpenicillin, and isolates are available for further laboratory testing. Exclusion • ASSOP patients not deemed PSSA by the treating institution

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	• ASSOP PSSA-B patients treated with other antimicrobials as definitive treatment.
Study Procedures	1. De-identified data from ASSOP categorized as “PSSA” and definitive antibiotic treatment “benzylpenicillin” or “flucloxacillin” and 30-day mortality. 2. If isolates are available, retrieve isolates for phenotypic testing with the EUCAST and CLSI methods for penicillinase detection, and presence of *blaZ.* 3. Statistical analysis conducted. (TBC after consultation with statistician)
Statistical Procedures	Logistic Regression Model and Chi Square test, TBC after consultation with statistician.
Duration of the study	Full calendar years of ASSOP: 2013-2021