Epidemiology, management and consequences of infection: a nephrology perspective

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Abstract

Healthcare associated infection confers a significant burden of morbidity and mortality to renal patients and to renal dialysis patients in particular. Sepsis is second only to cardiovascular disease as the leading documented cause of death in patients requiring renal replacement therapy. Gram positive bacteraemia is common in the renal replacement therapy population and is highly associated with indwelling haemodialysis catheter use. Optimal prevention and management of bacteraemia in this setting has not been fully determined and requires a multidisciplinary and multifaceted approach.

Each of the studies in this thesis investigates an aspect of healthcare associated infection in nephrology within the theme of exploring clinical problems arising from the development of antibiotic resistance or antibiotic associated infections in renal patients.

Initially we examined risk factors and outcomes of acute kidney injury requiring renal replacement therapy in a tertiary renal unit and critical care population prior to and subsequent to a change in antimicrobial guidelines in response to an outbreak of *Clostridium difficile* associated disease. We performed this study to address concerns that the increase in the empiric use of gentamicin may have led to an increased incidence of acute kidney injury and a greater requirement for emergency renal replacement therapy.

Secondly we explored the clinical implications of gram positive infection in a renal unit population by performing a retrospective review of *Staphylococcus aureus and* coagulase negative staphylococcal bacteraemia over a 2 year period with particular attention to admission rates, vascular access intervention, antibiotic resistance, metastatic infection and mortality.

Thirdly we have analysed *S. aureus* toxin genes and assessed the epidemiology of *S. aureus* colonisation and infection to improve our understanding of the virulence of *S. aureus* in different patient populations including a large haemodialysis unit in Glasgow.

Finally we undertook a prospective double blind randomised controlled trial of probiotic milk drink and placebo in renal unit inpatients commencing antibiotic therapy to assess if a probiotic was effective in the prevention of antibiotic associated diarrhoea and *Clostridium difficile* associated diarrhoea. We performed this study as patients with chronic kidney

disease are at increased risk of infection and have a significant antibiotic burden, which can lead to antimicrobial resistance, antibiotic associated diarrhoea and pseudomembranous colitis due to *Clostridium difficile* infection.

The study of healthcare associated infection is an evolving field and involves complex interactions between colonisation and infection. There is increasing emphasis on prevention of infection and minimising complications and side effects associated with standard antimicrobials. The rising incidence of multiresistant bacterial infections is likely to result in increasing focus on preventive bundles of care and alternatives to antimicrobial therapy such as the use of probiotics. The findings of this thesis contribute to the goal of prevention of antibiotic resistance and multiresistant infections in renal patients although further research is required.

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Author's declaration

The work presented in this thesis is that of the author and her supervisors, Dr Robert Mactier and Professor Thomas Evans. All clinical research work was carried out by the author, with the exception of some patient recruitment and data collection by Sister Elizabeth Bell (Chapter 5 only).

All statistical analyses were carried out by the author.

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I declare that this thesis has been composed by myself, and is a record of work performed by me. It has not been previously submitted for a higher degree.

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Publications

Gentamicin and acute kidney injury requiring renal replacement therapy in the context of a restrictive antibiotic policy. Helps, A., Deighan, C., Gourlay, Y., Seaton, RA. J Antimicrob Chemother. 2011 Aug;66(8):1936-8. doi: 10.1093/jac/dkr177. Epub 2011 May 24

Poster presentations

A retrospective study of Staphyloccus spp. Bacteraemia in a renal unit. Helps A., Marek A., Deighan C., Thomson P., Coia J. Federation of Infection Societies/ Healthcare Infection Society Conference, 19th-21st November 2012, Liverpool

Observational study of the prevalence of *Staphylococcus aureus* toxin gene positivity in samples from different patient populations including a renal dialysis unit in Glasgow, UK. Dr A Helps, Dr G Edwards, Dr R Mactier, Professor J Coia. European Renal Association/EDTA Conference, 18th-21st May 2013, Istanbul

Definitions/Abbreviations

ACE	Angiotensin converting enzyme
AAD	Antibiotic associated diarrhoea
ARB	Angiotensin 2 receptor blocker
AKI	Acute kidney injury
ARF	Acute renal failure
AVF	Arteriovenous fistula
AVG	Arteriovenous graft
BBE	Bare below the elbows
BMI	Body Mass Index
BURP	Based Upon Repeat Pattern
CA-MRSA	Community acquired methicillin resistant *Staphylococcus aureus*
CDAD	*Clostridium difficile* associated disease
CFU	Colony Forming Units
CI	Confidence Interval
CKD	Chronic Kidney Disease
CRBSI	Catheter-Related Blood Stream Infection
CRP	C-Reactive Protein
CRRT	Continuous renal replacement therapy
CVC	Central Venous Catheter
CVVH	Continuous veno-veno filtration

CVVHDF	Continuous veno-veno diafiltration
eGFR	Estimated Glomerular Filtration Rate
EPR	Electronic Patient Record
ESRF	End-stage renal failure
ETA	Exfoliative toxin A
ETB	Exfoliative toxin B
GGC	Greater Glasgow and Clyde
HCAI	Healthcare associated infection
HD	Haemodialysis
HPS	Health Protection Scotland
HR	Hazard Ratio
ITU	Intensive Treatment Unit
IRRT	Intermittent renal replacement therapy
KDIGO	Kidney Disease Improving Global Outcomes
KIM	Kidney Injury Molecule
HCAI	Healthcare associated infection
HD	Haemodialysis
HD	Haemodialysis
HPS	Health Protection Scotland
HR	Hazard Ratio
ICU	Intensive Care Unit
IRRT	Intermittent renal replacement therapy

KDIGO	Kidney Disease Improving Global Outcomes
MRSA	Methicillin Resistant *Staphylococcus Aureus*
MSSA	Methicillin Sensitive *Staphylococcus Aureus*
NCEPOD	National Confidential Enquiry into Patient Outcome and Death
NGAL	Neutrophil gelatinase-associated lipocalin
NTCVC	Non-Tunnelled Central Venous Catheter
OR	Odds Ratio
PCR	Polymerase chain reaction
PPI	Proton pump inhibitor
RIFLE	Risk, Injury, Loss, Failure, Endstage renal disease
RRT	Renal Replacement Therapy
SAB	*Staphylococcus aureus* bacteraemia
SD	Standard Deviation
SLED	Sustained low efficiency dialysis
SSS	Scalded skin syndrome
TSS	Toxic shock syndrome
TSST	Toxic shock toxin

Chapter 1: Background

1.1 Acute kidney injury

1.1.1 The evolution of acute kidney injury as a diagnosis

Identification of an acute decline in renal function was first mentioned as a clinical entity in the 18th century although Hippocrates correctly identified that the presence of bubbles in the urine could indicate renal disease in the 4th century BC. In the 2nd century AD, Galen of Pergamos first observed that blood is filtered by the kidneys and urine is transported to the bladder by the ureters. He identified a basic differential diagnosis of a reduction of urine output based on the presence or absence of a distended urinary bladder based on clinical examination (1). Later that century, Rufus Ephesius studied the changes associated with initial oliguric then polyuric acute renal dysfunction. Non oliguric acute renal dysfunction had not been recognised. By the 4th century AD, it was well established that if oliguria or anuria persisted, then death would follow. Treatment was supportive with dietary measure and improved sanitation although laxatives were sometimes used in a primitive method of water and toxin removal.

The 19th century English physician Richard Bright first connected the historical illness of "dropsy", observed as a clinical triad of widespread oedema, pathological renal abnormalities at postmortem examination, and proteinuria (found by heating the urine and denaturing the protein) (2). He published detailed drawings of dissected kidneys with granular changes under the renal capsule with loss of some anatomical landmarks and also described pericardial effusions and cerebral haemorrhage. It is thought that these descriptions and drawings are the first visual representations of glomulonephritis. Bright's disease may be the first regularly used English eponymous disease. Later, Richard Bright was involved in the identification of elevated blood urea levels in Bright's disease. He also describes abnormalities in the pulse of patients with Bright's disease that are felt to represent hypertension, which had not yet been identified as devices measuring blood pressure came into use in the 1890s.

Military medicine in the 20th century resulted in more detailed pathological examination of the diseased kidney following trauma and crush injury leading to the identification of pigmented casts and tubular damage, potentially as a result of rhabdomyolysis. This was

initially termed “war nephritis”. The term “acute renal failure” was first introduced by Homer W Smith in his textbook “The Kidney: Structure and Function in Health and Disease” (2). Knowledge progressed during the remainder of the 20th century with the development of haemodialysis, renal transplantation and further pathological diagnoses, however a clinical definition of acute renal failure and formal diagnostic criteria remained elusive. This resulted in wide variations in the reported incidence of acute renal failure and heterogeneity of studies leading to difficulty with comparison between populations and huge variations in reported clinical outcomes. The first haemodialysis machine was built by Williem Kolff in the Netherlands in 1944 (3). He used cellophane tubing as a conduit to carry the patient's blood through an extracorporeal circuit in contact with an electrolyte bath of known composition, equivalent to the concentration of electrolytes and glucose in normal plasma. Comments made in these early experiences of what at the time, was termed “ lower nephron nephrosis“ remain pertinent to the management of a patient with AKI today:

> *“Clinical management of acute renal insufficiency is usually difficult and at times discouraging but a majority of patients will respond to conservative measures. The causes of death in the remaining minority are pulmonary edema, extreme uremia, fulminating potassium intoxication and overwhelming sepsis from infection usually introduced at the time of the original trauma, which in turn precipitated the lower nephron nephrosis. Conservative therapy with special emphasis on proper hydration of each individual patient (scrupulously avoiding overhydration) is the keystone of the therapeutic arch.”*

1.1.2 Defining acute kidney injury

Evidence from the early 21st century demonstrated that small rises in serum creatinine associated with acute illness resulted in significant increases in mortality(4). As a result, the term ARF was replaced with acute kidney injury (AKI). The RIFLE criteria were developed by the Acute Dialysis Quality Initiative (AQDI) in order to incorporate the spectrum of the clinical syndrome (risk, injury, failure, loss, end stage kidney disease (ESRD)) (5). This pyramid of diagnostic criteria are illustrated below (Figure 1-1)

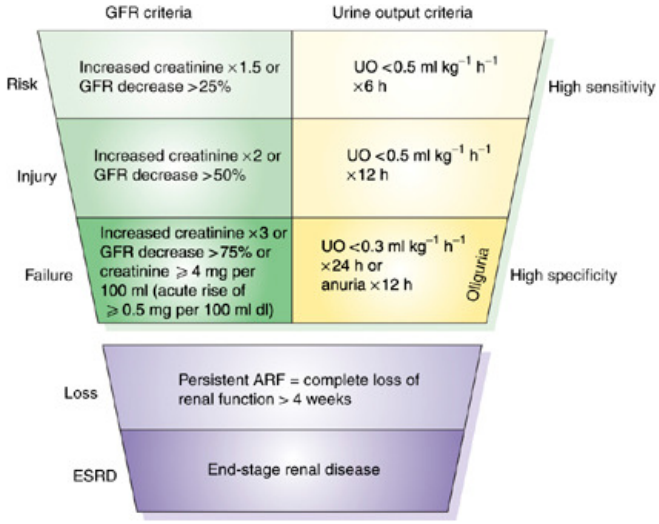


Figure 1-1 The RIFLE classification separates criteria for serum creatinine and urine output (5)

The severity of AKI is graded based on changes in serum creatinine or urine output with the worst of each criterion used. A retrospective observational study of over 8000 intensive care unit inpatients showed clear separation by 60 days survival according to the RIFLE criteria with the difference persisting to 1 year (Figure 1-2). Progressing through the RIFLE stages is associated with increasing length of stay in the ICU and hospital and decreased renal recovery(6).

The RIFLE criteria were limited by its requirement for retrospective information and a baseline serum creatinine. Accurate urine output monitoring can be difficult out of a critical care setting and can be affected by diuretics and abnormalities in ADH (anti diuretic hormone) secretion such as diabetes insipidus.

The RIFLE criteria have been applied to various patient groups including burns (7), decompensated heart failure (8), and in brain deceased kidney donors where the risk and injury groups were associated with delayed graft function (9).

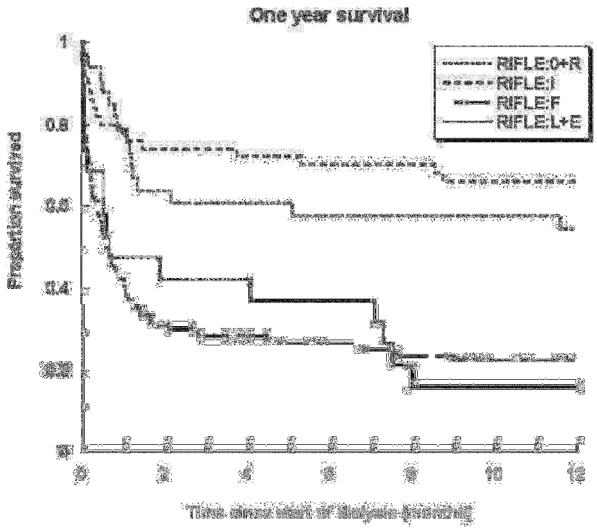


Fig. 2. One-year survival after stratification with the RIFLE criteria.

Figure 1-2 One-year survival after stratification with the RIFLE criteria (6)

Recognition of AKI and stratification of its severity was further refined by the Acute Kidney Injury Network (AKIN). Their criteria were based on the RIFLE criteria and first proposed in 2007. The major addition was the broadening of the "risk" category of RIFLE to include smaller changes in serum creatinine. Change in serum creatinine was to be documented over a 48 hour window. A statement was added that criteria were to be used after "optimum hydration and easily reversible causes were excluded".

Both the RIFLE and AKIN criteria have been used in several large trials and are well validated tools in predicting prognosis in AKI although evidence of superiority of AKIN over RIFLE is lacking (10).

Initially, AKI staging criteria were predominantly used in research and audit or in highly monitored environments such as the intensive treatment unit (ITU), however, increasingly they have been identified as triggers for specific forms of clinical assessment or investigations or as part of early warning scoring systems.

Most recently, the KDIGO have further refined diagnostic criteria with the staging criteria as defined below (Table 1-1). It is hoped that this will translate to improved outcomes and reduction in severity of AKI.

Table 1-1 KDIGO staging criteria for severity of AKI

Stage	Serum creatinine	Urine output
1	1.5-1.9 times baseline OR ≥ 26.4micromol/l increase	<0.5ml/kg/h for 6-12 hours
2	2.0-2.9 times baseline	<0.5ml/kg/h for ≥ 12 hours
3	3.0 times baseline OR Increase in serum creatinine to ≥ 353.6micromol/l OR Initiation of renal replacement therapy	<0.3ml/kg/h for ≥ 24 hours OR Anuria ≥ 12 hours

Any staging criteria for AKI require initial recognition that the patient is suffering from or at risk of AKI and appropriate alteration in their management with regular monitoring of biochemistry, fluid balance and clinical assessment.

1.1.3 Recognition of AKI

Despite the now well known increase in mortality associated with AKI, recognition and appropriate management of patients with AKI can be difficult. The National Confidential Enquiry into Patient Outcome and Death (NCEPOD) held an enquiry into management of patients who died at least in part secondary to AKI and identified deficiencies in management of this patient group. (11) A total of 587 patients had case notes and questionnaires returned to the reviewers. 90% of patients included had been admitted to hospital as an emergency and 60% of patients were under the care of general or medicine for the elderly physicians. 88% patients had evidence of kidney disease on admission with 46% of these patients being diagnosed with AKI.

The NCEPOD team attempted to evaluate by means of retrospective case note review, whether care of such patients was adequate and identified several areas of deficiency including lack of recognition of AKI, delayed management and in some cases, lack of referral to tertiary services when in retrospect this was appropriate. They detailed a number of main recommendations relating to prompt checking of electrolytes on emergency admissions, early senior review of all acute admissions, appropriate access to nephrology and critical care settings and 24 hour access to imaging and emergency urinary tract decompression of urinary tract obstruction. The NCEPOD review also stressed the requirement for robust assessment of the patient who acquires AKI while in hospital and that there is a means of recognising and highlighting the acutely unwell patient and instituting an appropriate plan for review and monitoring as recommended in the National Institute for Clinical Excellence (NICE) guideline 50.

Overall, only 50% patients were considered to have experienced good clinical care. A much smaller proportion, (<10%), were considered to have had deficiencies in organizational aspects of their admission. Only 30% of those who developed AKI while inpatient in hospital were considered to have experienced good quality care. 64% patients had a definitive diagnosis made to explain the episode of AKI. 43% of patients who developed AKI while an inpatient had an unacceptable delay in the recognition of AKI in the opinion of the advisors. 20.6% cases of AKI occurring while inpatient were determined as being predictable and avoidable in the opinion of the assessors. In total, 60% patients were in stage 3 AKI when their renal failure was recognised. 113 patients were transferred to renal units or critical care. Forty-four of the 273 remaining patients with available case notes who were not transferred to a renal unit or critical care setting were judged as potentially benefiting from transfer to a higher level of care.

Access to nephrology advice has been identified as impacting on the level of care a patient with acute kidney injury receives. The NCEPOD found that 14% hospitals did not have access to an on call nephrologist for telephone advice.

The NCEPOD report concluded that deficiencies in the management of a patient with AKI are likely to be representative of deficiencies in the general management of the acutely unwell hospital inpatient.

Timing of admission to hospital has also been found to impact on treatment for AKI (12). Retrospective database analysis of 963,730 admissions with a diagnosis of AKI within

acute care, nonfederal U.S. hospitals found that 22.3% patients were admitted at a weekend. They had similar baseline characteristics and length of stay, however there was significantly increased odds ratios for death when adjusted for age, gender, race, Charlson comorbidity index, and use of mechanical ventilation at 3 days; 1.22 (1.15 to 1.30) and in hospital; 1.07 (1.02 to 1.12). This large dataset is limited by its retrospective data where AKI was identified by means of clinical coding. These findings from a large clinical database highlight the need for further investigation of the availability of senior clinicians and diagnostic services outwith normal working hours.

1.1.4 Timing of renal replacement therapy in acute kidney injury

There is no consensus on the optimum time to commence renal replacement therapy (RRT) for AKI. Indications for emergency RRT are well established, however the exact timing is controversial. It is conventional to initiate RRT when oliguria persists despite correction of precipitating factors in order to prevent and treat the complications of AKI, including hyperkalaemia, fluid overload, metabolic acidosis and symptomatic uraemia. Timing of RRT in the non oliguric patient is less obvious with no definite benefit to early initiation of RRT or well conducted studies in this area.

Observational research has suggested a U shaped curve between the timing of the initiation of RRT and in hospital mortality and that staging criteria in this context is unhelpful. The heterogenous nature of patients requiring acute RRT results in difficulties in comparison between patients and study groups (13) (14). Meta analysis is also limited due to variations in parameters used in the initiation of RRT although the lack of a specific trigger such as serum creatinine or urea has been highlighted(15).

The only randomised controlled study investigating the impact of the timing of RRT on outcomes originated in the Netherlands where 106 patients in an intensive care unit (ICU) setting were randomised to early versus late initiation of RRT. The early initiation group started RRT within 12 hours of oliguria or at a creatinine clearance (CrCl) of 20ml/min. The late-initiation group started RRT when classic indications were met. There was no significant difference in ICU or in hospital mortality, and no difference in renal recovery although it is limited by its small size (16).

1.1.5 Mode of renal replacement therapy in acute kidney injury

The indications for emergency renal replacement therapy are well recognised, being refractory hyperkalaemia, metabolic acidosis, fluid overload resistant to medical therapy, symptoms or signs of uraemia and toxicity with certain poisonous substances (17, 18). Each modality has advantages and evidence of superiority of one modality over another in the acute setting is lacking in part due to the heterogenous nature of the populations involved. A Cochrane review found that there was limited evidence of continuous veno veno filtration resulting in a more stable mean arterial pressure in haemodynamically unstable patients although this has not been shown to translate into improved mortality (19). The optimum dose of renal replacement therapy in AKI is also yet to be determined. There is no convincing evidence of a mortality benefit to high doses of renal replacement therapy (20).

RRT in the acute setting can be delivered in a variety of different environments and modes. Haemodialysis (HD) is predominantly delivered in a nephrology setting. HD has the benefit of providing intermittent RRT allowing the patient to mobilise and does not require systemic anticoagulation. It allows the diffusion of solutes across a semipermeable membrane. The dialysate flows in the opposite direction to blood in the extracorporeal circuit, thus maintaining the concentration gradient and increasing dialysis efficiency. Ultrafiltration results from an alteration of the hydrostatic pressure across the membrane allowing free water to move out of the blood compartment.

Continuous veno veno haemofiltration (CVVH) is delivered in a critical care setting. In CVVH there is movement of solutes across a semi permeable membrane using convection which are then drained with isotonic fluid added to the resulting blood to replace water and solutes. There is limited evidence that CVVH provides greater haemodynamic stability, however evidence of superiority of one mode of RRT against another is lacking with similar outcomes reported (19) (18).

Continuous veno veno haemodiafiltration (CVVHDF) is a combination of HD and CVVH. Blood is pumped through the blood compartment of a high flux dialyser with a high rate of ultrafiltration, resulting in the movement of water and solutes from blood to dialysate. These are replaced by substitution fluid that is infused directly into the blood line.

However, dialysis solution is also run through the dialysate compartment of the dialyser. The combination is theoretically useful because it results in good removal of both large and small molecular weight solutes. It is increasing used in an ESRF population although hard outcome data suggesting benefit is currently lacking. It was available in 50% critical care units in 2008 and used first line in 16% of units (21). Intermittent haemodiafiltration was compared to intermittent HD in a small randomised non blinded study of 39 patients with acute kidney injury with similar outcomes and biochemical parameters (22). CVVHDF and intermittent HD were compared in a randomised controlled trial of 360 patients in French intensive care units between 1999 and 2003 (23). There was no significant difference in death between the 2 groups with survival at 60 days being 32% in the intermittent HD group and 33% in the CVVHDF group (95% CI -8.8 to 11.1). There was significantly more hypothermia in the CVVHDF group (p=0.0005).

A hybrid technique named sustained low-efficiency dialysis (SLED) is employed in some units providing RRT to critically ill patients. The first published data was from a single centre in Arkansas, USA where 145 SLED treatments were performed in 37 patients where intermittent HD had failed due to intradialytic hypotension, failure to meet solute clearance goals or been withheld as a result of predicted haemodynamic intolerance by the treating clinician (24). The inpatient mortality of 62.2% was not significantly different to that predicted by APACHE II scores at admission to the ICU or at initiation of RRT. Retrospective database analysis comparing haemodynamically unstable patients who received CVVH between January 2002 and January 2004 and those who received SLED between February 2004 and August 2006 has suggested that mortality was improved in the SLED group. This has the disadvantage of a retrospective observational analysis with those receiving SLED requiring RRT 2 years after the initial cohort with significant difference in inotrope use and creatinine level at initiation of RRT. An economic advantage to SLED has been suggested when a prospective randomised study designed to compare clinical outcomes between SLED and CVVH showed significantly reduced nursing and consumables costs associated with SLED saving €1300 per patient (25). There was no significant difference between the pre-specified outcome measures and in particular, no difference in mortality between these 2 groups.

Peritoneal dialysis can be used in an AKI setting, however this is extremely uncommon in the adult environment in the UK, although is utilised in the Middle East and developing countries. (26) (27) (28)

1.1.6 Mortality associated with acute kidney injury

Prior to the development of the RIFLE criteria, mortality associated with AKI was not clearly defined due to differing definitions between studies. Although the RIFLE criteria were developed in order to standardise AKI diagnosis and severity rather than predict prognosis, systematic review has suggested a strong relationship between AKI severity and mortality with progression through the stages of the RIFLE criteria associated with increasing relative risk of death. It is notable that the populations studied are almost exclusively ITU patients as shown below in the forest plot below (Figure 1-3).

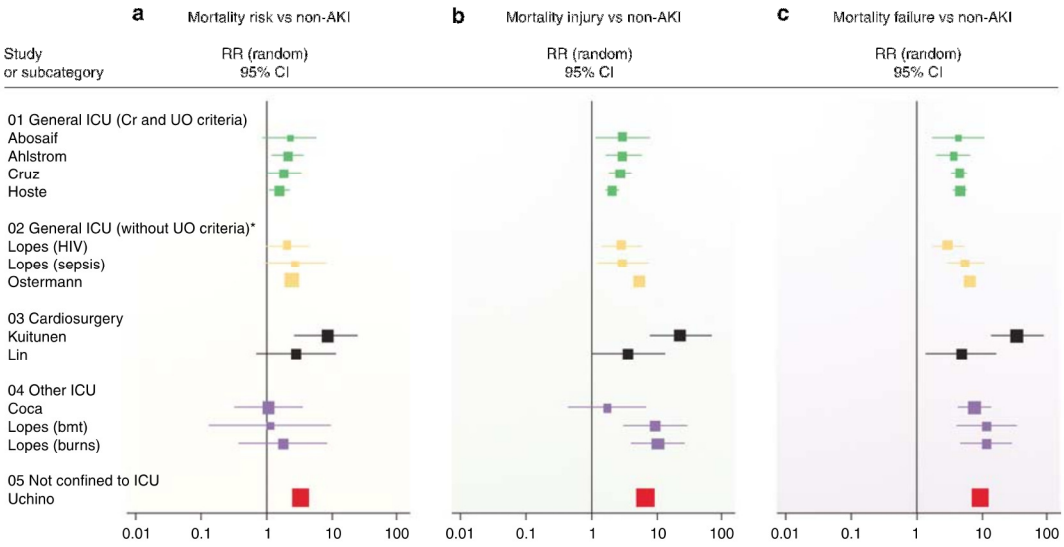


Figure 1-3 Forrest plot showing RR for death with respect to non-AKI patients (29). (a)Risk (RR 1⁄4 2.40; 58 073 participants included in meta- analysis), (b) Injury (RR 1⁄4 4.15; 55 351 participants included in meta-analysis), and (c) Failure (RR 1⁄4 6.37; 53 758 participants included in meta- analysis). Cr, creatinine; UO, urine output.

AKI after myocardial infarction comparing the RIFLE and KDIGO criteria was studied retrospectively in 1050 patients (30). AKI defined by RIFLE and KDIGO occurred in 14.8% and 36.6% of patients respectively. There was a significant association between patients with AKI as defined by either criteria and mortality at 30 days and at 1 year. Patients diagnosed with AKI with the KDIGO criteria but not RIFLE criteria also had significantly increased mortality ($p<0.001$). Patients were not stratified by stage of AKI. 38% and 26% of patients with AKI identified by RIFLE and KDIGO criteria respectively died within 30 days compared to 8% and 4.7% of the cohort not identified to have AKI by RIFLE and KDIGO criteria respectively.

Evaluation of 101 patients with acute myocarditis and preexisting normal renal function recorded in the National Taiwan University Hospital Study Group on Acute Renal Failure database (NSARF) found that, AKI defined as AKIN stage 3 and elevated Sequential Organ Failure Assessment score were independent risk factors of in-hospital mortality using multivariate logistic stepwise regression (31). Decreased left ventricular ejection fraction and elevated cardiac enzymes were not associated with an increased risk of mortality.

Retrospective analysis of patients with diffuse proliferative lupus nephritis suggested that the RIFLE criteria could predict short-term prognosis of AKI in this population (32). Patients at a more advanced stage of AKI were less likely to achieve complete renal recovery and more likely to have progressive renal impairment. The short follow-up duration of 24 weeks, lack of data on baseline serum creatinine, no availability of activity index from renal biopsy and the small sample size of 79 patients limit the study.

A prospective study of 200 patients newly diagnosed with high grade haematological malignancies compared remission rates in those with and without AKI as defined by the RIFLE criteria (33). 68.5% patients developed AKI. 91.4% of cases were as a result of hypoperfusion, tumour lysis syndrome, acute tubular necrosis, nephrotoxic agents, or hemophagocytic lymphohistiocytosis. 50% of the AKI patients required RRT and 14.6% received sub optimal chemotherapy. AKI was associated with a significantly lower 6 month complete remission rate (39.4% vs. 68.3%, $P<0.01$). The proportion of patients achieving complete remission fell with progression through the RIFLE stages.

Electronic results reporting identified AKI in a hospital-wide prospective study of AKI incidence over a 9 month period (34). This allowed data collection on AKI incidence and outcomes in the UK in an unselected hospital inpatient population albeit in a tertiary referral centre. 3202 AKI episodes in 2619 patients were identified using the AKIN diagnostic criteria. This represented 5.4% of hospital admissions (both elective and non elective). Of these patients, 435 had >1 episode of AKI and 1970 (61.5%) episodes were classified as stage 1 AKI, with similar numbers in stage 2 (638; 19.9%) and stage 3 (594; 18.6%). The in-hospital mortality rate for the entire AKI population was 23.8% (624 patients) with hospital wide mortality rate for emergency admissions at 3.2%. Patients with normal pre-existing renal function had increased risk of mortality as they progressed through the AKI stages. Significantly higher mortality rates were observed in those with hospital-acquired AKI: 28.9% compared to 20.6% in those with community-acquired AKI

(P<0.001). This study is notable due to the broad spectrum of patients included and large patient population with prospective data collection.

1.1.7 Outcomes in patients following AKI

Renal outcomes in an adult ICU population of 2164 patients were evaluated in the North East Italian Prospective Hospital Renal Outcome Survey on Acute Kidney Injury (NEiPHROS-AKI) trial (35). 10.8% patients developed AKI as defined by the RIFLE criteria. 19% were classified as risk (R), 35% as injury (I), and 46% as failure. 3.3% of all patients required RRT. Overall mortality for patients with AKI was 36.3%, significantly higher than the remainder of the cohort (p<0.001). Patients with class F AKI had a mortality of 49.5%. 36% of patients with AKI recovered renal function by the time of death or ICU discharge. No data was available for renal recovery in survivors.

Health-related quality of life (HRQOL) was assessed in 397 patients following ICU admission (36). 73 patients required RRT for AKI. Patients or a proxy completed the Short-Form 36 (37) within 48 hours of ICU admission and followed up survivors for 6 months following ICU discharge. Although patients had significantly lower HRQOL after ICU admission, there was no significant difference between patients who required RRT and those who did not.

A population based cohort study evaluated AKI outcomes from a provincial claims registry in Alberta, Canada with patients having been admitted to the hospital between November 1, 2002 and December 31, 2007 (38). 3.7% participants (7014 patients) experienced AKI during the study period. AKI in this study was defined as an increase in serum creatinine by ≥100% and/or requirement for acute dialysis during the index hospitalisation. 4400 patients (62.7%) survived 90 days after hospital discharge. 3231 of these patients were available for follow up for a median of 34 months. 30.8% of these patients died and 2.1% (85 patients) progressed to requiring long term renal replacement therapy. Baseline renal function was lower in patients who developed AKI (p< 0.01). Patients with complicated diabetes and congestive cardiac failure were less likely to recover renal function (p<0.01), as were patients with CKD at baseline (p<0.01). Patients who did not fully recover renal function had increased hazard ratios for death at 1.23 (95% CI 1.08, 1.40), which persisted when adjusted for age and comorbidities.

Meta analysis of AKI outcomes in 2009 included 49 studies although only 15 of these reported data relating to non-AKI controls (39). The incidence rate of mortality was 8.9 per 100 person-years in survivors of AKI and was 4.3 per 100 patient-years in survivors without AKI (RR 2.59, 95% CI 1.97-3.42). AKI was associated independently with mortality risk in 6 of 6 studies that performed multivariate adjustment (adjusted RR 1.6-3.9) and it was associated with myocardial infarction in 2 of 2 studies (RR 2.05, 95% CI 1.61-2.61). The incidence rate of CKD after an episode of AKI was 7.8 per 100 patient years and the rate of ESRD was 4.9 per 100 patient-years.

1.1.8 Economic impact of AKI

The cost of renal replacement therapy varies depending on the environment it is delivered (renal unit v critical care), staffing, consumables and treatment modality. The Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) study performed a post-hoc analysis of costs associated with RRT (40). Overall costs were not quoted. Nursing costs were higher with intermittent RRT (IRRT), however dialysate, extracorporeal circuits, and replacement fluid costs were higher with continuous RRT (CRRT). There was significant variation between centres and when all costs were combined cost differences ranged between $3629.80 more per day with CRRT to $378.60 more per day with IRRT. RRT for AKI in the Belgian multi-centre Stuivenberg Hospital Acute Renal Failure 4 study cost on average €30 447 for CRRT and € 25 176 for IRRT (41).

1.1.9 Gentamicin associated AKI

Gentamicin is the most widely used of the aminoglycoside antibiotics and has synergistic antimicrobial activity against gram negative bacilli and gram positive cocci. In line with other aminoglycosides, it has the potential for nephrotoxicity and ototoxicity. 99% of aminoglycoside administered parenterally is excreted via the kidney (42).

The precise pathophysiology of gentamicin-associated nephrotoxicity is not completely understood. It is well recognised that after filtration, gentamicin is absorbed into the proximal convoluted tubule by endocytosis leading to fusion with lysosomes and myeloid body formation, and movement into the golgi apparatus with movement into the endoplasmic reticulum before being released into the cytoplasm (43) (44). Thereafter, a

number of mechanisms for aminoglycoside-induced nephrotoxicity have been postulated. Various studies have identified renal abnormalities including decreased protein synthesis, induction of apoptosis, tubular necrosis and abnormal cell respiration although the relative importance of each is yet to be determined (45) (46) (47) (48) . Gentamicin-associated AKI is characteristically non-oliguric before glomerular filtration falls. Tubular regeneration marks renal recovery.

The reported incidence of gentamicin associated AKI varies due to differences in study design and definition. A historical review of available literature in 1984 found that 14% patients experienced gentamicin associated AKI although this is limited by a lack of consensus on the definition of AKI. Various risk factors for gentamicin-associated AKI have been identified, including abnormal baseline renal function (49), liver disease (50) and frequent dosing interval (51). The optimal dosing regimen is yet to be determined.

A retrospective observational study of gentamicin-associated AKI defined by the RIFLE criteria included all patients treated with gentamicin over a 1 month period. 24.4% patients had AKI defined by the RIFLE criteria although only 2.4% patients reached the “Failure” category. There was increased risk of in hospital mortality in patients with AKI, rising with progression through the RIFLE stages ($p<0.001$) (52).

High cumulative gentamicin dose increases the risk of development of AKI with prolonged duration of therapy and elevated plasma drug concentration being associated with deterioration in renal function (53) (54). Repeated short courses of gentamicin have also been shown to be nephrotoxic (54). It is unclear whether elevated peak or trough gentamicin levels are most toxic to the kidney. Increased age may result in inaccurate estimation of creatinine clearance and inadvertent excessive dosing.

Severe sepsis increases the risk of aminoglycoside toxicity. Volume depletion as a component of sepsis reduces the effective circulating volume resulting in renal ischaemia. Endotoxin is also directly toxic to the proximal convoluted tubule resulting in aminoglycoside accumulation (55).

Patients with advanced liver disease are a particular subset at high risk for gentamicin-associated AKI. They have reduced effective circulating volume with activation of the renin-angiotensin-aldosterone system (50). Obstructive jaundice gives increased risk of

gentamicin-associated AKI via unknown mechanisms (56). Hypoalbuminaemia is an independent risk factor for AKI (57).

The data presented in Chapter 2 adds to the existing body of research suggesting that gentamicin is a safe component in the management of sepsis provided there is sufficient monitoring and antimicrobial stewardship. The subset of patients with RRT dependent AKI is under represented in existing literature.

1.1.10 Pathophysiology of acute kidney injury in sepsis

The underlying causative mechanism of acute kidney injury in sepsis has not been fully determined. Histological information is limited as renal biopsy is potentially hazardous and impractical in such patients. The majority of historic animal models were based on ischaemia-reperfusion models, however small studies of larger animals suggest an increase in renal blood flow in the context of sepsis associated AKI(58). Monitoring of renal blood flow during an observational study of 8 septic intensive care unit inpatients found normal renal blood flow in the presence of acute kidney injury(59). Inflammatory cytokines have also been implicated. Renal endothelial dysfunction in sepsis is suggested by albuminuria indicating loss of integrity of the glomerular filtration barrier(60). There is evidence that mice deficient in tumour necrosis factor are resistant to the effects of lipopolysaccharide associated acute kidney injury with the absence of morphological changes in endothelial cell fenestration(61).

Programmed cell death or apoptosis is provoked by brief periods of renal ischaemia, however the role of apoptosis in sepsis associated AKI is unclear. Human proximal tubule cells underwent apoptosis when treated with tumour necrosis factor, interleukin 1 and lipopolysaccharide in one model of sepsis associated acute kidney injury(62). Apoptosis of renal cells was also induced when incubated with plasma from a rabbit model of acute respiratory distress syndrome suggesting interaction with other organ systems affected in the critically ill(63).

1.1.11 Role of urinary biomarkers in acute kidney injury

The identification of acute kidney injury prior to a rise in serum creatinine has been the subject of considerable research. Urinary biomarkers have been identified as non invasive indicators of acute kidney injury and can identify high risk patients, particularly in the context of a specific potential cause of AKI such as surgery or iodinated contrast administration(64) (65).

The urinary biomarkers neutrophil gelatinase–associated lipocalin (NGAL) and kidney injury molecule (KIM) have been used to identify undifferentiated patients with impending AKI in the accident and emergency department. NGAL is a small secreted polypeptide. It has been shown to be upregulated in the proximal tubular cell after an episode of renal ischaemia(66). KIM is a transmembrane protein. It is not usually expressed in renal tissue, however is present in high numbers in renal tissue following ischaemic or toxic injury(67). Urinary NGAL had increased sensitivity and specificity in one large study of over 1600 patients compared to other urinary biomarkers. Patients with elevated urinary biomarkers and AKI were found to be at increased risk in hospital mortality and requiring renal replacement therapy(68). There is also evidence that the underlying cause of AKI affects urinary NGAL concentration with a small study showing that patients with AKI secondary to sepsis had significantly increased urinary NGAL levels. There were the potential confounders of increased burden of illness and increased prevalence of malignancy in the sepsis group(69).

Despite promising results from observational studies, randomised controlled trials of the application of urinary biomarkers of AKI in clinical practice are lacking. The only published randomised controlled study evaluating the effects of intervention based on urinary biomarkers was terminated early due to adverse events in both study arms(70).

1.2 Role of *Staphylococcus* spp. bacteria in infection and disease

1.2.1 Taxonomy of staphylococcal bacteria

The term staphylococcus is from the Greek work *staphyle* meaning bunch of grapes due to the tendency of staphylococci to form microscopic grape-like clusters. They were first identified by Koch in 1878 who recognised that diseases such as abscesses correlated with the presence of clusters of Gram-positive cocci.

The *Staphylococcus* spp. and *Streptococcus* spp. bacteria constitute the family of Gram-positive cocci. The genus *Staphylococcus* are members of the Micrococcacae family. Over 60 species and subspecies of *Staphylococcus* are currently recognised (71). At least 18 staphylococcal species have been isolated from human skin although *S. epidermidis* accounts for at least half of the bacterial burden (72).

Gram-positive refers to the violet staining of the bacterial wall on Gram staining due to presence of a thick peptidoglycan layer in the cell wall. Staphylococcal bacteria are ubiquitous in their presence on the skin and mucosal surfaces of almost all animals including humans. *S. aureus* and the *S. hyicus-intermedius* group produce coagulase. *S. intermedius* are zoonotic organisms which can be associated with human disease (73). *S. aureus* causes more severe disease and can be distinguished genetically by the presence of the coagulase gene, clinically by its characteristic appearance and golden pigmentation, confirmed by latex agglutination testing and increasingly, using polymerase chain reaction testing (PCR) to detect the presence of the thermonuclease gene *nuc*.

Relationships between staphylococcal species can be estimated by DNA analysis using single or multiple genes and illustrated by a phylogram evolutionary tree as shown in Figure 1-4 below (71).

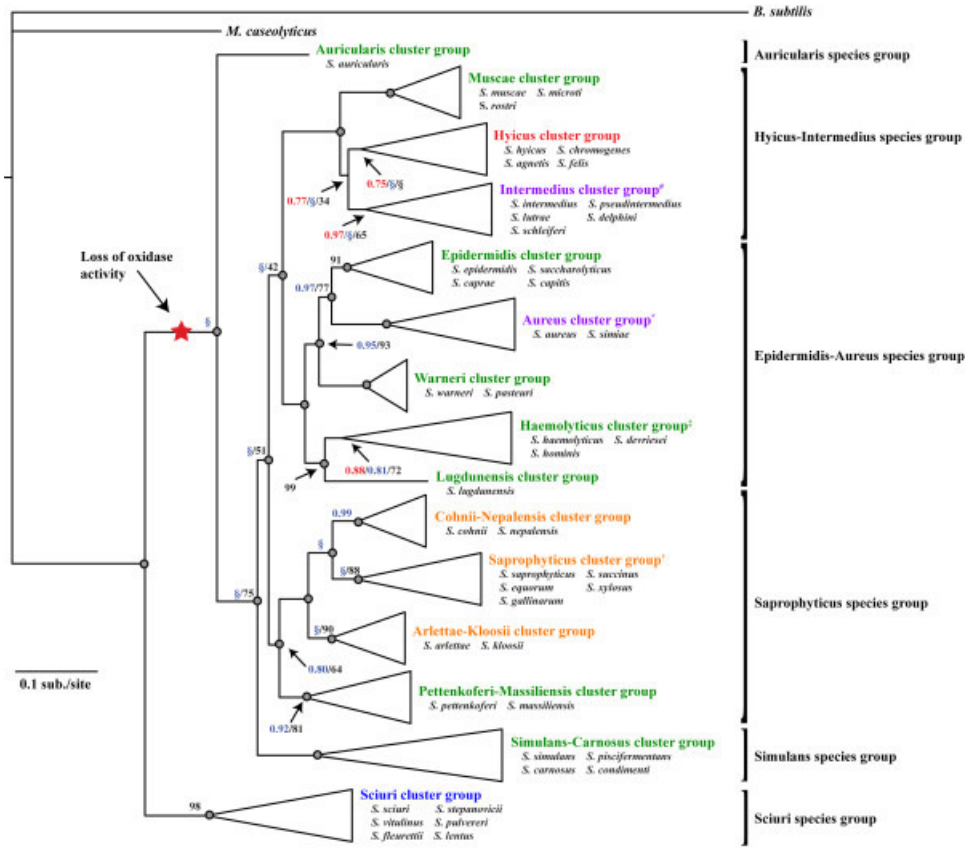


Figure 1-4 Summary phylogram showing Staphylococcal species combined into six species groups and 15 cluster groups. Cluster groups have been colour-coded to represent: blue, species that are novobiocin resistant, coagulase negative, and oxidase positive; green, species that are novobiocin susceptible, coagulase negative, and oxidase negative; orange, species that are novobiocin resistant, coagulase negative, and oxidase negative; purple, species that are novobiocin susceptible, coagulase positive, and oxidase negative; and red, species that are novobiocin susceptible, coagulase variable, and oxidase negative. Colour scheme exceptions are: #S. schleiferi schleiferi is coagulase negative; *S. simiae is coagulase negative; ‡S. hominis novobiosepticus is novobiocin resistant; and †S. equorum linens is novobiocin susceptible.

1.2.2 Role of S. aureus colonisation in disease

S. aureus is persistently present in the anterior nares of 30% of the population and transiently found in 70% of the population. There is evidence that there is increased nasal carriage of methicillin resistant *S. aureus* (MRSA) in those with frequent contact with domesticated animals, which suggests that they may be vectors in its spread (74). Although *S. aureus* shows preference for the anterior nares, there is an increase in carriage rates at extra-nasal sites in nasal *S. aureus* carriers. People colonised with community acquired MRSA (CA-MRSA) in particular, may be colonised in the throat or skin but not

the anterior nares (75). This forms the basis of MRSA screening of multiple sites in hospital inpatients.

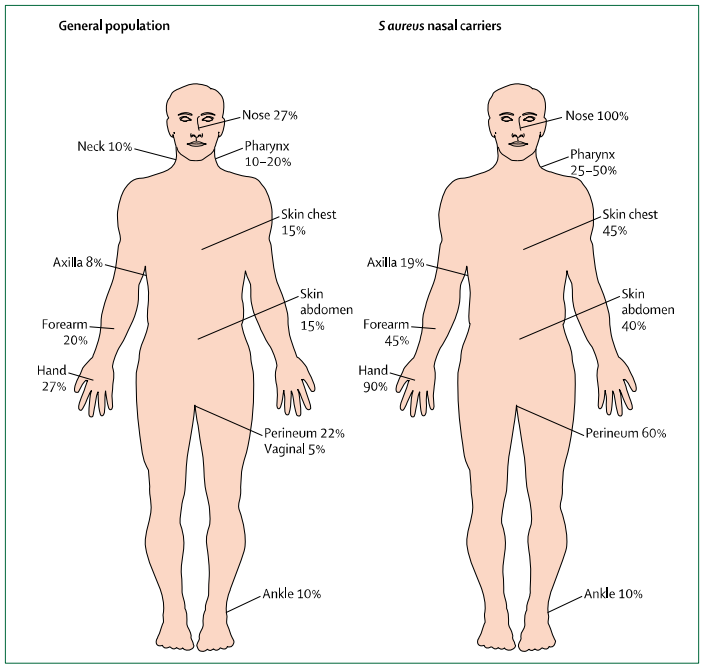


Figure 1-5 *S. aureus* carriage rates per body site in adults (76)

Carriers of *S. aureus* have increased risk of infection secondary to the *S. aureus* strain they carry. Rates of *S. aureus* carriage in healthcare workers are similar to adults in the general public. Longitudinal studies suggest that only around 20% people are persistent carriers of *S. aureus*. These individuals have a higher total burden of *S. aureus* on their skin (77). Studies of persistent carriers after attempted decolonisation suggest that they will select out their previous strain even after artificial inoculation with alternative strains (78). Observational studies show that insulin dependent diabetics and patients requiring renal replacement therapy (RRT) are more likely to be carriers of *S. aureus* (79) (80). There is also an association with *S. aureus* carriage and increased risk of relapse with ANCA-associated vasculitis with granulomatosis (previously termed Wegener's granulomatosis) (81) although there is no evidence that *S. aureus* eradication reduces the risk of relapse. Environmental factors cannot fully explain the variation in carrier states in humans with a genetic component being postulated. Evidence from twin studies is conflicting (82) (83).

S. aureus infection occurs either by self inoculation or by direct or indirect contact. Common risk factors *for S. aureus* infection include prior colonisation, poor hygiene, intravenous drug abuse and comorbidities such as chronic skin conditions, cystic fibrosis

and renal dialysis. The density of nasal colonisation and associated secondary colonisation of the skin appears to be important in the risk of staphylococcal infection.

There is specific evidence that elimination of the *S. aureus* carrier state in some populations may reduce the incidence of infection, however the role of *S. aureus* colonisation eradication in populations with a long-term increased risk of *S. aureus* infection, such as dialysis patients is less clear. Any eradiaction therapy utilising antimicrobials such as mupirocin has the potential to promote resistance. Bacterial interference may be a more attractive option where the pathogenic *S. aureus* is replaced with an avirulent version (84).

Attention to hand hygiene is important in reducing healthcare-associated infection including the transmission of *S. aureus*. Compliance with hand hygiene can be notoriously low. A large longitudinal observational study found hand hygiene rates increasing from 48% to 66% over a 3-year period with an associated fall in MRSA colonisation and nosocomial infection (85). This observation is correlation rather than causation in this population of hospitalised patients. There were other confounding variables during this time period including variations in antimicrobial prescribing.

The evidence for the relationship of hand hygiene and nosocomial infections was reviewed further with 31 original studies identified by 2008 on systematic review (86). Over half of these originated from the USA. Deficiencies in study methodology were identified with the lack of a control group being raised as a particular issue. The authors of this systematic review concluded that research linking hand hygiene and HCAI is present, but it has not been fully quantified.

The “bare below the elbows” advice in the prevention of HCAI has been criticised due to its minimal evidence base. One study randomized 157 doctors and medical students to “bare below the elbows” (BBE) or conventional dress (non BBE) with a white coat tailored to the carpometacarpal joint of the thumb (87). There was no significant difference in the percentage area of the hands missed during hand washing between the BBE group and the non-BBE group ($P = 0.281$) (BBE mean: 9.3% ± 9.2; non-BBE mean: 11.1 ± 7.2%). The non-BBE group missed significantly more of the wrist compared with the BBE group ($P = 0.002$). The mean percentage area missed on the wrists in both groups was significantly higher than on the hands ($P < 0.001$). The authors recognised that the act of observation may have influenced participants although this is likely to be similar in both

groups. BBE therefore had no discernable effect on the effectiveness of hand washing. The significance of improved wrist washing with BBE is difficult to translate into improved clinical outcomes against HCAI. Wristwatch wearers have also been shown to have an increased bacterial load on their wrists but not their hands in contradiction with standard infection control advice (88).

1.2.3 The environment and bed occupancy in S. aureus transmission

Hospital environment, high bed occupancy and patient movement between clinical areas may increase transmission of bacteria in a hospital or healthcare trust wide model (89) (90) (91) (92) (93). Unfavourable staff-to-patient ratios have been associated with decreased hand hygiene and overcrowding of the ward area has also been associated with an increase in MRSA transmission and infection despite an increase in beds available, perhaps due to higher bacterial contamination of surface areas with increased patient density.

The patient environment is a common reservoir for bacteria. Hygiene on 2 UK surgical wards was monitored by weekly screening of 10 hand-touch sites during an observational study (94). *S. aureus* was isolated from lockers, overbed tables and beds. Recovery of *S. aureus* at any site was significantly associated with aerobic colony counts of > 2.5 cfu/cm^2 which in turn were significantly associated with weekly bed occupancy >95% ($p = 0.0004$; OR: 2.94 [95% CI 1.44, 6.02]). This was true for both methicillin sensitive *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA).

This observation was taken further by an intervention study where a cleaner was introduced with the sole job specification of cleaning of common hand-touch sites such as those discussed above (95). Two wards were included with each ward receiving enhanced cleaning for six months in a cross-over design. Ten hand-touch sites on both wards were screened weekly and patients were monitored for methicillin-resistant *S. aureus* infection throughout. Molecular analysis assessed the relationship of any MRSA infection to that found in the environment. Enhanced cleaning was associated with a 32.5% reduction in levels of microbial contamination at hand-touch sites when wards received enhanced cleaning ($P < 0.0001$: 95% CI 20.2%, 42.9%). The two wards were well matched for demographics with the exception of Ward A being predominantly male and Ward B female. There were no changes to hospital antimicrobial policy or to MRSA screening

policy during the period studied. The authors found that there were fewer new MRSA infections during enhanced cleaning at 26.6% (95% CI 7.7%, 92.3%) of the rate during normal cleaning ($P = 0.032$). Molecular epidemiological methods supported the possibility that patients acquired MRSA from hand-touch sites.

1.2.4 Active surveillance of MRSA colonisation

The screening for MRSA and subsequent isolation of colonised individuals is a widespread practice in the healthcare setting. The National MRSA Screening Programme utilises a Clinical Risk Assessment (CRA) designed to identify high risk individuals. All admissions with the exception of obstetrics, paediatrics and psychiatry are assessed by the 3 questions:

- Has the patient ever had a previous positive MRSA result?
- Has the patient been admitted from a care home/institutional setting or another hospital?
- Does the patient have a wound/ ulcer or invasive device that was present prior to admission?

If the answer to any of these questions is yes, the patient undergoes a full MRSA screen. This involves swabbing of the anterior nares and perineum in addition to skin lesions, wounds and invasive devices if there are any signs of infection. High impact specialities including critical care, orthopaedics, nephrology, vascular and cardiothoracic surgery are exempt from the CRA and patients require MRSA screening regardless of any other risk factors.

A 12-month prospective trial of universal screening for MRSA in acute care in Scottish hospitals found that 3.39% patients (2717/69 445 patients) were identified as colonised with MRSA on admission (96). The initial colonisation prevalence of 5.5% significantly decreased to 3.5% by month 12 of the study ($P < 0.0001$). Multivariate analysis found that elderly patients, those with more than a single hospital admission during the year, those admitted from another hospital, care home or institution, in addition to those admitted under medicine, orthopaedics, ICU, care of the elderly or oncology were more likely to be colonised with MRSA. A total of 422 MRSA infections were identified during the study period. It is notable that the odds of developing an MRSA infection among patients who screened positive on admission and who were previously known to be positive for MRSA

colonisation was 18.3 (95% CI: 13.0–25.9) times greater than those screened negative for MRSA. The odds of developing infection for those who were screen positive on admission and who were not previously known positive were 11.9 (95% CI: 8.1–17.6) times greater than for those who screened negative. These data suggests that universal screening for MRSA may be of benefit, especially in hospital settings with a high overall prevalence of MRSA. It would be important to consider the resource implications of this before such a programme is implemented (97).

1.2.5 MRSA

The number of penicillinase-producing *S. aureus* was very low in the 1940s and this rapidly increased when penicillins were introduced. Methicillin (the first penicillinase resistant **β**-lactam antibiotic) was introduced in 1959 and the first MRSA strains appeared 2 years later and were rapidly established in places of high antibiotic use. The European Antimicrobial Surveillance System (EARSS) monitors MRSA prevalence in European countries. It maintains a comprehensive surveillance and information system that provides comparable and validated data on the prevalence and spread of major invasive bacteria such as MRSA.

Methicillin resistance is conferred due to the acquisition of the staphylococcal chromosomal cassette *mecA* gene (SCC*mec*). This is confirmed by polymerase chain reaction (PCR) testing. The coagulase negative *Staphylococcus epidermis* is frequently methicillin resistant and carries SCC*mec* genes with associated theoretical interspecies transfer of this gene to *S. aureus*.

1.2.6 MRSA and MSSA bacteraemia surveillance and monitoring

It is mandatory to report all cases of *S. aureus* bacteraemia to Health Protection Scotland (HPS). Health Protection Scotland was established by the Scottish Government in 2005 to strengthen and co-ordinate health protection in Scotland. It is organised into three specialist groups: Healthcare Associated Infections and Infection Control; Blood Borne Viruses and Sexually Transmitted Infections, Immunisation, and Respiratory and Vaccine Preventable Diseases; Gastrointestinal and Zoonoses, Travel, and Environment and Health.

HPS has published quarterly reports on the numbers of MRSA bacteraemias in Scotland since April 2002. The programme was extended to include reporting of MSSA bacteraemias from July 2006.

Renal units accounted for nearly 5% of all epsodes of methicillin sensitive *Staphylococcus aureus* (MSSA) bacteraemia between 1 April 2005 to 31 March 2009 and 5.5% methicillin resistant *Staphylococcus aureus* (MRSA) bacteraemia between 1 January 2003 to 31 March 2009 in Scotland (98). It is clear that central venous catheters are a specific risk factor for bacteraemia in such patients and have been associated with an increased risk of mortality (99).

The HEAT targets are well defined as:

- **H**ealth Improvement for the people of Scotland - improving life expectancy and healthy life expectancy
- **E**fficiency and Governance Improvements - continually improve the efficiency and effectiveness of the NHS
- **A**ccess to Services - recognising patients' need for quicker and easier use of NHS services
- **T**reatment Appropriate to Individuals - ensure patients receive high quality services that meet their needs

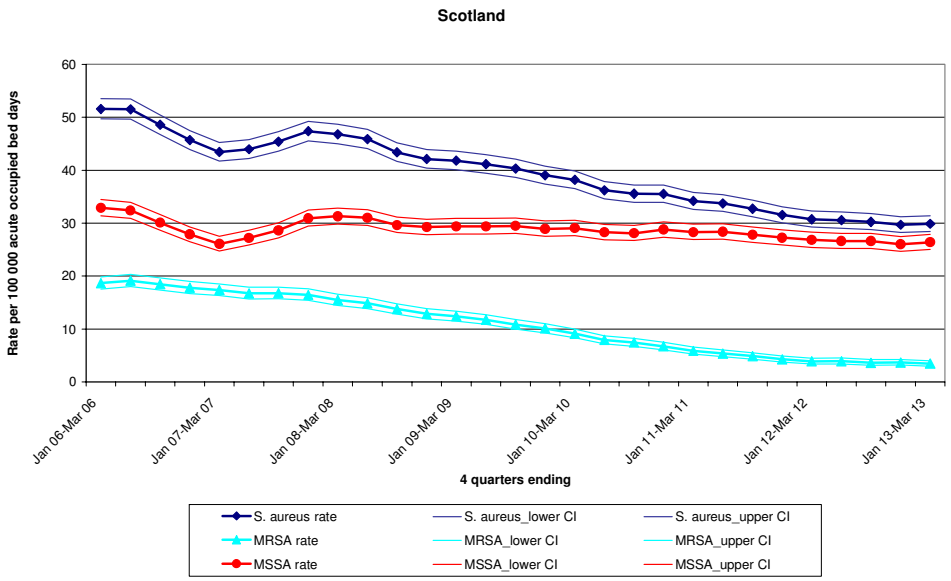


Figure 1-6 MRSA and MSSA bacteraemias per 100 000 acute occupied bed days in Scotland from March 2006 until March 2013 (100).

Figure 1-6 above shows national performance on MRSA and MSSA infections from March 2006 until March 2013. For the quarter ending July 2013, the rate of MRSA/MSSA cases across NHS Scotland was 0.31 per 1,000 acute occupied bed days. The current HEAT target is to further reduce SAB (including MRSA) cases to 0.24 or fewer per 1000 acute occupied bed days by March 2015. Although MRSA cases have consistently fallen, MSSA bacteraemia rates are static and efforts to reduce MSSA bacteraemia will be required for this target to be reached.

There appear to be differences in the patient populations affected by MSSA and MRSA bacteraemia (101). MRSA bacteraemia is associated with 2.2 fold increased mortality, increased duration of hospital admission and increased healthcare costs. MRSA bacteraemia in HD patients is 100x that of the general population (45.2 vs 0.4 episodes/1,000 patient-years) (102). HD patients account for up to 15% of all invasive MRSA infections in the USA.

Vancomycin resistant S. aureus (VRSA) remains very rare, with only a few cases reported worldwide. The first European case of VRSA was reported in a Portuguese diabetic HD patient in July 2013(103).

1.2.7 Staphylococcus aureus virulence and pathogenicity

S. aureus possesses a number of mechanisms which facilitate its persistence in the environment and enhance its ability to cause disease. The accessory gene regulator (*agr*) reacts to bacterial density, allowing the bacteria to shift between a growth phase in areas of low bacterial density, to a stationary phase where it expresses virulence genes in order to enhance pathogenicity. The staphylococcal accessory regulator gene (*sar*) encodes a DNA-binding protein, which controls *agr.* Inactivation of *agr* and *sar* have been shown to decrease the pathogenicity of *S. aureus* in experimental models.

Some *S. aureus* clones have the ability to form biofilm although this is more of a feature of the less pathogenic coagulase negative staphylococci. The polysaccharide capsule of the *S. aureus* bacteria can increase *S. aureus* virulence by being anti-phagocytic and is a potential target for vaccines against *S. aureus*. Enzymes secreted by *S. aureus* are often proteases

and lipases which promote bacterial pathogenicity by causing tissue destruction and providing nutrition to the invading bacteria.

The role of the toxin genes including the Panton Valentine Leucocidin toxin in causing disease is discussed in Chapter 4.

1.2.8 Coagulase-negative staphylococcal disease

The coagulase-negative staphylococci are the most abundant microbes on normal human skin and mucous membranes. They are mostly regarded as non-pathogenic commensals. However, they have been associated with disease, particularly in nosocomial infections involving implanted vascular devices, prosthetic joints and prosthetic heart valves. *S. epidermis* is most commonly isolated in humans and is the most prevalent species on human skin with the average person carrying more than 10 different strains. *S. lugdunesis* is rarer, but can result in a clinical syndrome more in keeping with *S. aureus* infection and therefore requires more aggressive treatment. *S. epidermidis* is responsible for 50-70% of catheter associated infections caused by coagulase negative staphylococci(104). This has been attributed to its ability to form biofilm. Different species of coagulase negative staphylococcus have varying antimicrobial susceptibility, virulence and adhesion properties and therefore cause differing patterns of disease(105). An example is *S. saprophyticus,* which can account for up to 10-15% urinary tract infections with increased prevalence in young women(106). A mechanism for this is a capacity to bind preferentially to uroepithelial cells(107).

Coagulase negative staphylococci are often found as components of a biofilm on intravascular catheters and this is implicated in its mechanism of causing disease. It binds to the surface then forms an extracellular matrix before forming a “biofilm community”. In this environment, the bacteria are in varying states of replication from quiescence to active replication.

Despite the ubiquity and persistence of coagulase negative staphylococci, native valve endocarditis due to coagulase negative staphylococci is uncommon, accounting for 5-8% cases although this rises to 20% of prosthetic valve endocarditis cases. It remains the most common cause of intravascular device infection although it tends to give a less severe clinical course than *S. aureus.*

1.2.9 Staphylococcal bacteraemia in renal patients

Renal dialysis patients are at high risk of *S. aureus* bacteraemia (SAB) due to their frequent contact with healthcare services and the high proportion of haemodialysis (HD) who have indwelling central venous catheters. Even patients receiving RRT via arteriovenous fistulae (AVF) are at a higher risk of bacteraemia than the general population.

The risk of bacteraemia in HD patients changes according to the form of vascular access with AVF conferring the lowest risk of bacteraemia, followed by arteriovenous grafts (AVG) and tunneled central venous catheters (TCVCs) and non-tunnelled central venous catheters (NTCVC) conferring the highest risk of bacteraemia (99). The site of dialysis catheter is also important with internal jugular NTCVCs imparting a lower risk of bacteraemia compared to NTCVCs sited in the femoral vein (99). NTCVC insertion into subclavian vein has been found to have a lower incidence of infection compared to internal jugular vein (108). However, insertion of catheters into the subclavian vein tends to be avoided due to the considerable risk of angiographic stenosis following catheter insertion at this site (109).

A bundle of measures are employed in an effort to reduce the risk of infectious complications associated with central venous catheter (CVC) insertion. "Maximal sterile barriers" were employed in an effort to reduce CVC related infection in a 500 bed cancer referral centre (110). 176 patients were randomized to maximal sterile precautions consisting of mask, cap, sterile gloves, gown and large drape and compared to 167 control patients who received standard precautions at the time consisting of sterile gloves and small sterile drapes. Patients were followed up for 3 months or until the catheter was removed. There was a significant fall in catheter related infection in the group randomised to maximal sterile barriers with a total of 4 catheter infections in the test group and 12 in the control group ($p=0.03$). It is impossible to know which of the several interventions above provided most impact on the reduction of CVC related infection. It is notable that the study population were not patients requiring renal replacement therapy.

1.2.10 Antimicrobial line locks

Catheter lock solutions have been shown to reduce the incidence of catheter related bloodstream infection (CRBSI) in some settings although there is no consensus for their use in routine clinical practice (111) (112). Antibiotic line locks have been used with some effect with gentamicin, vancomycin, minocycline, cefazolin and cefotaxime all being used either alone, in combination with another antibiotic or in combination with another antimicrobial agent. The majority of studies included patients with TCVCs only. Alternative antimicrobials such as citrate and taurolidine have also been used to prevent CRBSI and these have the advantage of not promoting antibiotic resistance. The mechanism of action of an antimicrobial line lock is the limitation of biofilm formation and this may explain their effectiveness against coagulase negative staphylococcal bacteria in particular. Heparin was used as the control line lock in the majority of clinical studies and is the standard agent for the locking of TCVCs and NTCVCs. Some studies included additional measures such as povodine- iodine application at the catheter exit site or nasal mupirocin and as a result are difficult to compare directly.

1.2.11 Treatment of haemodialysis catheter related infection

Haemodialysis (HD) patients with CRBSI present a challenge due to their ongoing requirement for access to the intravascular tree. Any attempt made to salvage the HD catheter should be balanced against the risk of inadequately treated infection and metastatic complications and therefore management of CRBSI must be tailored to the requirements of the individual.

It is convention that the initial empirical antimicrobial in CRBSI is intravenous vancomycin(113, 114). Coagulase negative staphylococci commonly exhibit methicillin resistance, therefore vancomycin is a logical choice while a pathogen is cultured. Once a pathogen is cultured, the antibiotic therapy should be rationalized. If MSSA is cultured, the current recommendation is for therapy to be altered to flucloxacillin in the UK, or cefazolin in the USA. Cefazolin utilizes intermittent dosing which allows outpatient treatment in the HD unit and has been shown to provide a mortality benefit in MSSA compared to vancomycin (115). Flucloxacillin is considered to have superior efficacy

against MSSA compared to vancomycin although research studies confirming this are lacking (116).

S. aureus bacteraemia (SAB) secondary to NTCVC insertion requires NTCVC removal. TCVCs should also be removed in the event of *S. aureus* CRBSI due to the risk of metastatic infection, which increases if TCVC removal is delayed. Removal of the intravascular device has been associated with more prompt resolution of infection and higher cure rate. It is recommended that repeat TCVC should not be reinserted until blood cultures are negative where feasible. Persistently positive blood cultures are a sign of haematogenous spread and should prompt further investigation. The optimum duration of antimicrobial therapy has not been the subject of a randomized controlled trial.

CRBSI secondary to coagulase negative staphylococcal infection characteristically gives rise to a less severe illness and therefore the HD catheter can often be salvaged. A NTCVC should still be removed where possible and replaced once blood cultures are negative for 72 hours. An exception to this is *Staphylococcus lugdunesis* bacteraemia which, despite being a coagulase negative *Staphylococcus*, gives rise to a clinical syndrome similar to SAB with a relatively high incidence of metastatic infection and therefore should be treated more aggressively (117). Diagnosis of CRBSI secondary to coagulase negative *Staphylococcus* is difficult in the absence of clinical signs as it is commonly viewed as a contaminant and depends on multiple positive blood cultures from multiple sites. The optimal duration of antimicrobial therapy in uncomplicated CRBSI secondary to coagulase negative *Staphylococcus* remains uncertain and evidence is based on expert opinion only. Antibiotic line locks have been used as an adjunct in the treatment of CRBSI with some success, particularly in the treatment of coagulase negative staphylococcal bacteraemia (118).

1.2.12 Typing of Staphylococcus aureus strains

The epidemiology of *S. aureus* infection relies on typing methods based on their genotypic characteristics. This helps to establish clonal relationships between strains and to trace the geographic dissemination of bacterial clones. Pulsed-field gel electrophoresis after SmaI digestion of total bacterial DNA was originally proposed for outbreak investigation was used widely(119) (120). It is time consuming and expensive therefore limiting its use in routine clinical practice.

Multilocus sequence typing relies on the sequences of the internal fragments of seven housekeeping genes(121). It is also expensive compared to *spa* typing. Spa typing is based on the sequence of an internal fragment of the *spa* gene, which is the polymorphic X region of the protein A gene on the cell wall of the *S. aureus* bacterium. The region consists of a variable number of repeat units with each new base composition of a repeat being assigned an alpha-numerical code. The repeat success determines the *spa* type(122). These DNA sequence-based typing methods have the benefit of generating unambiguous and portable data, allowing comparison to be made between different geographical areas(123).

Relationships between different *spa* gene polymorphisms are visually represented using Based Upon Repeat Pattern (BURP) diagrams. They are used to infer clonal relatedness from *spa* repeat regions(124) and therefore construct a population snapshot as illustrated below(124).

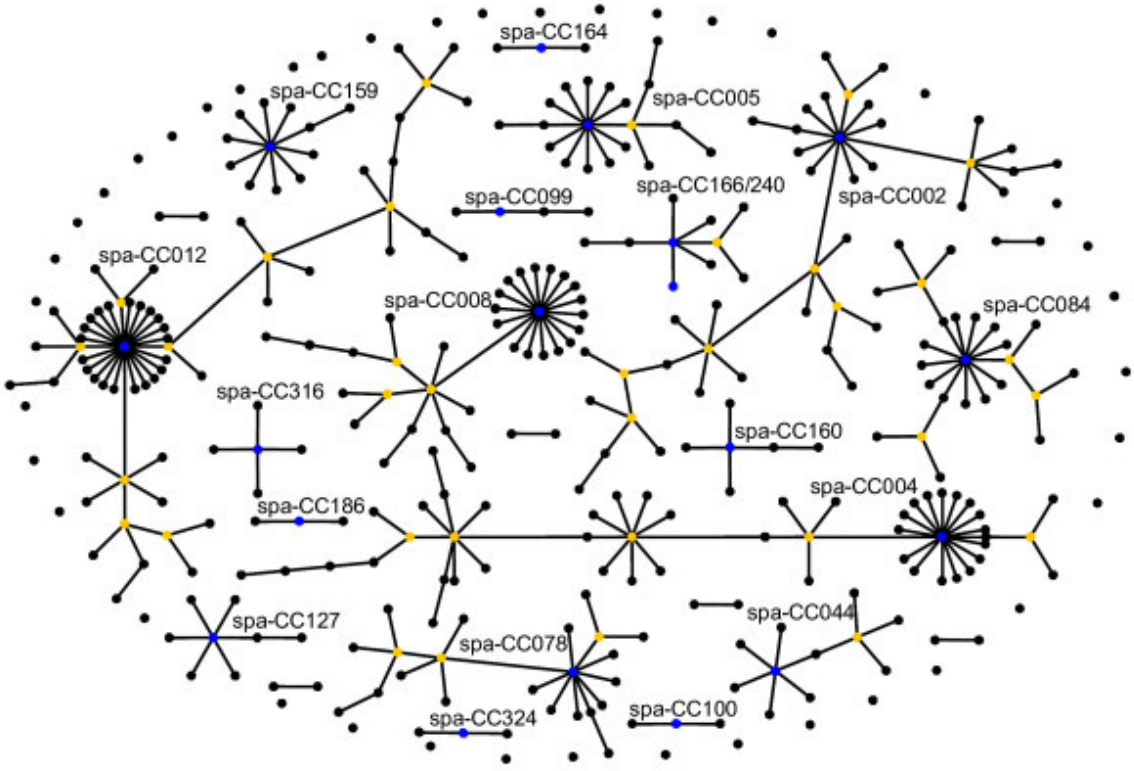


Figure 1-7 Population snapshot of the 400 *S. aureus* strains after BURP grouping (124)

The Discriminatory Power (D) is the average probability that the typing system will assign a different type to two unrelated strains randomly sampled in the microbial population of a given taxon. It con be expressed by the formula of Simpson's index of diversity which reads-

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} x_j(x_j - 1)$$

Where D is the index of discriminatory power, N the number of unrelated strains tested, S the number of different types, and xj the number of strains belonging to the jth type, assuming that strains will be classified into mutually exclusive categories. Thus, a D value of 1.0 would indicate that a typing method was able to distinguish each member of a strain population from all other members of that population. Conversely, an index of 0.0 would indicate that all members of a strain population were of an identical type. An index of 0.50 would mean that if one strain was chosen at random from a strain population, then there would be a 50% probability that the next strain chosen at random would be indistinguishable from the first(125) (126).

The data presented in Chapter 3 describes the incidence, management and outcomes of staphylococcal bacteraemia in renal inpatients with particular attention to treatment and outcomes in HD patients.

1.3 Antibiotic associated diarrhoea and probiotics

1.3.1 Development of the human intestinal microbiota

The human intestinal microbiota is absent at birth but rapidly develops over the first 2 years of life. The mode and location of delivery influences its development in the very early stages, however the sequence of colonisation with various bacteria is well established with initial colonisation with aerobic bacteria, followed by faculatitive anaerobes such as *enterobacteria* spp followed by aerobes including *bifidobacteria* spp. *Bifidobacteria* spp were first identified in the 1900s by Tissier, who linked their numbers in the faeces to the ability to resist bacteria and first discovered their presence in the stools of breast fed infants. It has been established that the intestinal microbiota is an important aspect of the immune system.

Germ free mice reared in sterile environments have a poorly developed immune system with defined immune deficiencies, reduced number and distribution of certain immune cells and failure to thrive. They carry no microorganisms in their intestines and body and therefore exposure to bacterial stimuli can be manipulated for research purposes. They have reduced numbers and size of Peyer's patches, decreased IgA secreting plasma cells, decreased lamina propria CD4+ T cell numbers and altered intra-epithelial T cell contents with abnormal cytokine production (127) (128). Ito found that when germ free mice were colonised with the flora of normal healthy mice in a process termed conventionalization, there were measurable improvements in their immune response (129).

The Nurmi concept took this further with "competitive exclusion" cultures selected from the mucosa of healthy birds given orally in order to prevent salmonella infection (130). They hypothesised that the abnormally hygienic environment of a poultry farm resulted in impaired development of the intestinal flora and therefore reduced resistance to infection. This was implicated in an outbreak of *Salmonella infantis* in humans with poultry acting as a reservoir of infection. They compared 1-2 day old chicks given 0.5ml gut contents from adult cocks diluted with 0.9% saline to control animals reared. The groups were reared separately and each bird was held in isolation. Both groups were given *S. infantis* and began a standard feeding regimen free from antibiotics and chemotherapeutics. Chicks were killed when they were 8-22 days old and bacteriological examination was performed. All chicks who did not receive gut contents were colonised with *S. infantis* compared to the chicks with pretreatment of adult gut contents where 23% of those given 10^3 *S. infantis* and 31% of those 10^6 *S. infantis* were colonised. This demonstrated the importance of the gut microbiota in the protection of the body against pathogenic bacteria.

Various mechanisms within the gut prevent the adhesion and translocation of pathogens. Gut-associated lymphoid tissue (GALT) is present throughout the gut, particularly in the small intestine. Peyer's patches are aggregates of lymphoid tissue located in the ileum. These are covered by a protective layer of mucus, which in addition to its barrier function, contains antimicrobial peptides originating from the commensal microbiota and the host. Abnormal functioning of the GALT can result in increased risk of infection and bacterial translocation. It also has a role in the pathogenesis of autoimmunity involving coeliac disease and inflammatory bowel disease

The intestinal microbiota affects the innate and acquired immune response using toll-like receptors expressed on epithelial cells, microfold cells (M cells) transporting bacterial and antigens from the gut lumen to lymphoid tissue and dendritic cells in the lamina propria sampling luminal antigens. All of these processes have been the subject of research with regards to the influence of probiotics on the gut microbiota and immune system.

A normal adult gut microbiota comprises 10^{14} microbes and greater than 1 kg bacteria. This results in a genome 150x the size of the adult human host with up to 1500 bacterial species with complex metabolic interactions and relationships. Bacterial colonisation density is greatest in the large bowel and least in the upper gastrointestinal tract as demonstrated below. Bacterial distribution depends on the available nutrients including oxygen in addition to gut transit time and pH. The most hospitable environment is the colon which contains more than 90% bacteria due to its low luminal oxygen concentration, slow transit time, less acidic pH and favourable nutrient availability.

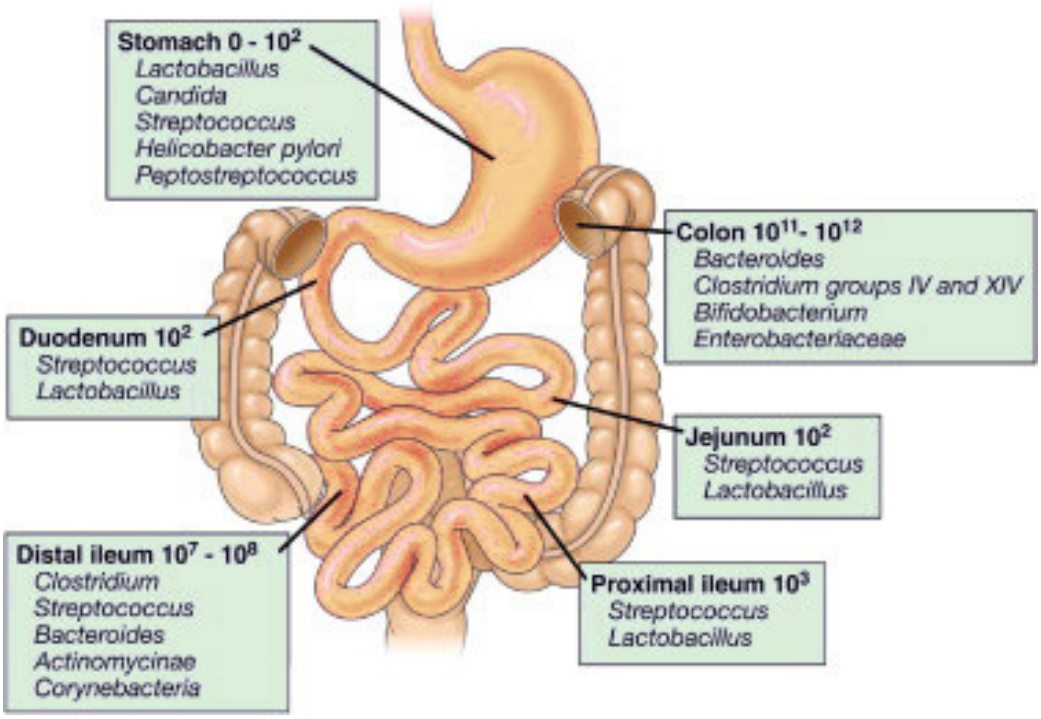


Figure 1-8 Distribution and abundance of bacteria in the human gastrointestinal tract (131)

The number of species comprising the intestinal microbiota result that some are likely to be beneficial to the host although some bacteria have obvious pathogenic potential. The beneficial activity of the intestinal microbiota are those bacteria with saccharolytic

properties rather than those that possess proteolytic properties or have putrefying activities. Disruption of the intestinal microbiota, for example, with antibiotic therapy, can increase disease susceptibility.

1.3.2 Role of probiotics and prebiotics in manipulation of the intestinal microbiota

Probiotics and prebiotics have been used in clinical research in an attempt to promote the integrity of the intestinal microbiota in health and when it is interrupted as a result of disease, in particular due to antibiotics or inflammatory bowel disease. Probiotics have been defined by the Food and Agriculture Organisation of the United Nations, (FAO), and the World Health Organisation (WHO) (132), as live microorganisms which when administered in adequate amounts confer a health benefit on the host. There is no necessity for these beneficial effects to be gut related. Prebiotics are non-digestible substances that when consumed provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (133). These can be dietary carbohydrates known to have a selective metabolism, for example, oligosaccharides and fructooligosaccharides. Synbiotics are less clearly defined but tend to be a combination of prebiotic and probiotic which may have synergistic properties.

Probiotics may exert a beneficial effect on the host via various mechanisms of action which are similar to that described above for the commensal gut bacteria. Specific immune effects are more likely to be strain specific whereas the effects of competitive exclusion for lactobacilli and bifidobacteria as they transit through the gut are likely to be more species specific.

In general, the gut effects of a probiotic are in the maintenance and restoration of the intestinal microbiota with specific effects on gut barrier function. They increase numbers of short chain fatty acids and decrease luminal pH. They also increase mucus production resulting in improved barrier function. More subtle effects on immune modulation in the gut and elsewhere are in the process of being determined and are not fully understood. There is some evidence of a therapeutic benefit of probiotics in the reduction of duration of upper respiratory tract infections, particularly in the elderly and in athletes (134) (135) (136), who have increased susceptibility to such infections although this is not a consistent

finding (137) (138). Mechanistic studies suggest that salivary IgA and natural killer cells may have a role here (139) (140) (141). There has also been investigation into the role of probiotics in functionally immunocompromised patients such as those at risk of ventilator associated pneumonia (142).

Synbiotics have been used in research into the reduction of postoperative infections, in particular, one small study of 50 living liver transplant recipients randomised to *Bifidobacterium breve, Lactobacillus casei*, and galactooligosaccharides or control resulted in a reduction in postoperative infections up to 2 weeks after surgery. This is limited by lack of blinding (143).

1.3.3 Clinical trials of prebiotics and probiotics

Probiotic use in critically ill patients must still be viewed with caution. Although there are case reports only of lactobacilli causing symptomatic bacteraemia, one large randomized controlled trial was discontinued early due to excess mortality in the treatment arm (144). Besselink *et al* recruited 298 patients predicted to have acute severe pancreatitis into a double blinded comparison of six different strains of freeze-dried, viable bacteria: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium bifidum*, and *Bifidobacterium lactis* against placebo. It was stopped early after an interim analysis showed excess mortality in the probiotic group shown in Figure 1-9 below. A follow-up of longer than 90 days was obtained in 266 (90%) patients. Three deaths occurred after 90 days: two in the probiotics group (day 112 and 125) and one in the placebo group (day 140).

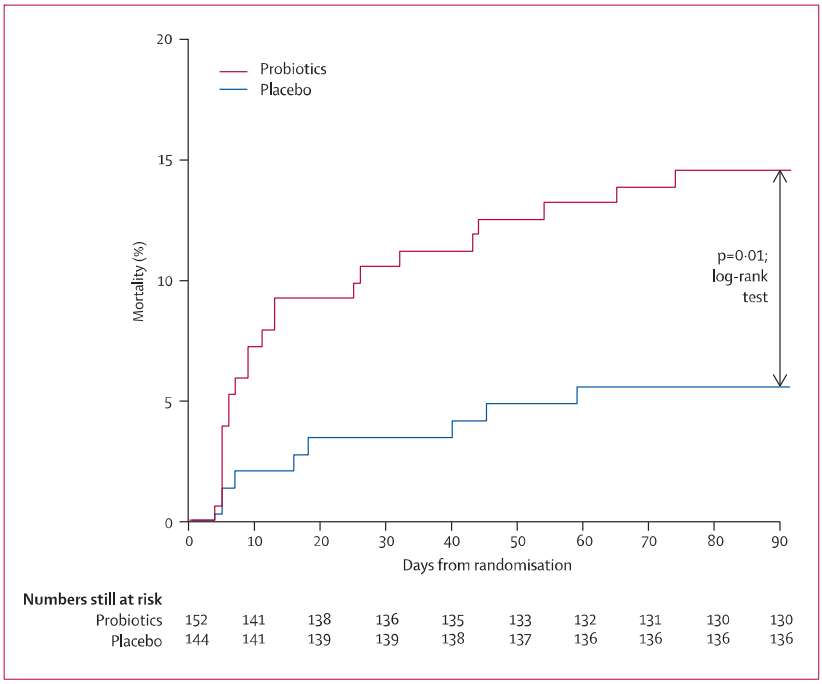


Figure 1-9 Kaplan–Meier time-to-event analysis for mortality in the first 90 days after randomisation

Relative risk for mortality in the probiotic group was 2·53 (95% CI 1·22–5·25). The majority of the deaths in each group were due to multi organ failure. There was bowel ischaemia at surgery or post mortem examination in 9 patients in the probiotic group with 8 of these patients dying as a result. There was no bowel ischaemia in the placebo group (p=0.004). It has been postulated that the mechanism behind the mesenteric ischaemia was increased enteric blood demand due to the presence of probiotic or metabolic stress of gut epithelial cells due to increased bacterial load.

It is estimated that between 5 and 39% patients experience diarrhoea as a consequence of antibiotic treatment depending on the population and type of antibiotic used. This can result in a more prolonged hospital admission and in the most extreme cases, *clostridium difficile* associated diarrhoea, which significantly increases morbidity and mortality.

The elderly are more susceptible to antibiotic associated diarrhoea. It has been postulated that a mechanism behind this is that the gut microbiota in the elderly are less efficient with evidence of immune senescence (145). There are changes in the composition of the intestinal microbiota with lower numbers of *bifidobacteria* and *bacterioides*, with decreased species diversity and increased numbers of *enterobactericae*. This was taken further by Van Tongeren in 2005 where they investigated the relationship between faecal microbiota composition and frailty in the elderly (146). They found that subjects with high

frailty scores had x26 reduction in lactobacilli with very frail subjects having a x7 increase in *Enterobacteriaceae (*facultative anaerobes).

Probiotics have been used with very limited success in the treatment of norovirus. There is a single open label case controlled study of *Lactobacillus casei* Shirota (*Lc*S) in elderly patients resident in a care home (147). There was a reduction in fever but no difference in diarrhoea and vomiting duration or incidence. Faecal acetic acid concentration before, during and after *Lc*S administration was measured and found to increase, and was correlated with increasing *bifidobacterium* concentration, however this is difficult to interpret in the clinical context of recent infectious diarrhoea.

1.3.4 Antibiotic associated diarrhoea and Clostridium difficile associated disease

The effect of even a short course antibiotic on the bowel flora in otherwise healthy patients can be significant. Dethlefsen *et al* investigated faecal bacteria as a surrogate for large bowel bacteria before and after a 5 day course of oral ciprofloxacin at its usual therapeutic dose (148). They found that there was a reduction in bacterial species with some strains not recovering within 6 months. This is limited study population of only 3 patients but has been followed up in larger scale studies, mostly investigating the effects of probiotic administration.

Antibiotics are known to disrupt the colonic microbiota by two main mechanisms. They reduce the metabolism of fermentable carbohydrates resulting in a reduction in the number of short chain fatty acids and increased amount of non-absorbable carbohydrate in the gut lumen, leading to osmotic diarrhoea. They also lead to a reduction in the resistance of the gut flora to the overgrowth of pathogenic bacteria such as *C. difficile, C. perfringens, Salmonella, S. aureus* and yeast such as *C. albicans* as a result of a reduction of competitive exclusion.

Clostridium difficile associated disease (CDAD) is the most severe form of antibiotic associated diarrhoea. *Clostridium* bacteria are rod-shaped spore-forming bacteria that live under anaerobic conditions. It was first discovered in 1935 (149) and first associated with disease in 1978 (150). It is an opportunistic pathogen and common environmental reservoirs include soil, water, hay, and sand. Eradication of *C. difficile* from the

environment is difficult because of its spore forming properties and that it can be carried asymptomatically in the bowel in approximately 3% of adults in health (151). This is significantly more after an insult to the intestinal microbiota by antibiotic therapy and in hospital inpatients.

Mortality associated with CDAD can be as a direct result of bowel perforation or surgery required for toxic megacolon or the more insidious effects of diarrhoea including poor nutrition, volume depletion and general increasing frailty. Studies of outcomes in CDAD are difficult to compare due to different end points with not all reporting absolute mortality. McGowan *et al* reviewed all patients diagnosed with CDAD in a specific UK NHS hospital trust between 2002 and 2008 and reported absolute mortality at 30 days of 32.7% (152). There was no significant difference between the study years or between hospitals. Increasing age, increasing comorbidity, renal impairment, hypoalbuminaemia, leukocytosis and leucopaenia have all been associated with increased risk of mortality in CDAD (153) (154) (155) (156) (157) (158).

Acute kidney injury (AKI) was found to be associated with adverse outcomes with a retrospective review of over 1 million patients entered into the National Hospital Discharge Summary database (159), which provides data on demographics, diagnoses, procedures, length of stay, discharge, and funding. They found that patients with CDAD and AKI had nearly 3 times higher all-cause in-hospital mortality on univariate and multiple variable logistic regression analysis in addition to increased risk of colectomy, length of hospital admission and increased likelihood for discharge to a facility providing further short or long-term care. An Austrian prospective cohort study of 185 patients admitted to a community hospital with CDAD found that 13% died during their hospital stay compared to 2.7% of controls. After adjustment for age, sex and co-morbidity the relative risk of pre-discharge death was 2.74 (95 % CI 1.82–4.10; $p < 0.0001$) for patients with CDAD (160). Furthermore, analysis of the same cohort suggested that patients with underlying chronic kidney disease (CKD) may also have increased risk of adverse outcomes although there was no significant difference in patients requiring renal replacement therapy (RRT) to the remainder of the CKD cohort.

Antibiotic use is intrinsically associated with *Clostridium difficile* associated disease (CDAD). Antibiotic choice is crucial in the reduction of *C. difficile* with some broad spectrum antibiotics resulting in an increased risk of CDAD compared to others as discussed in Chapter 5.

Increasing age is a risk factor for antibiotic associated diarrhoea and CDAD and the changes in the gut microbiota associated with increasing age have been discussed above. Proton pump inhibitor (PPI) use has been associated with antibiotic associated diarrhoea in multiple large studies (161) (162). These increase the gastric pH and can affect gut flora in many ways. Tanner *et al* investigated risk factors associated with CDAD in two UK hospitals and found an elevated Waterlow score to have a positive predictive value of 16.8% and negative predictive value of 99% (163). 33% of patients who developed CDAD (32/98) came from the 5% of patients with a Waterlow score of ≥20. The Waterlow score is a well-validated risk assessment tool for predicting pressure sore risk using several risk factors including body mass index (BMI), continence and mobility (164) (165).

A large American retrospective cohort study compared outcomes of 41 000 patients with CDAD to 4 000 000 propensity score matched controls (166). Average length of stay was longer for CDAD patients by 5 days ($p < 0.001$) with higher overall mortality albeit with small absolute differences (9.4% vs. 8.6%; $p < 0.001$). Hospital costs were 56% higher for CDAD patients with a mean hospital admission cost of $23 350 ($p < 0.001$).

CDAD occurs as a result of disruption of the protective bowel flora allowing *C. difficile* spores to germinate resulting in toxin damaging the epithelium. Injured epithelial cells result in the leakage of neutrophils and erythrocytes into the gut. Severe CDAD can result in pseudomembranous colitis. The pseudomembrane is as a result of a viscous collection of fibrin, inflammatory cells and necrotic epithelial cells. CDAD is pathognomonic of pseudomembranous colitis although very rarely it can also occur in diarrhoea caused by other bacterial infections. Toxic megacolon can occur and in some cases, colectomy is required.

A hypervirulent strain of CDAD termed NAP-1/ ribotype 027 was identified in 2005 (167) (168). It was associated with increased toxin and spore production and is highly transmissible. It has a higher mortality and is more difficult to eradicate. It has been associated with outbreaks in the healthcare setting and was associated with severe complications such as pseudomembranous colitis and toxic megacolon in addition to increased mortality and lower cure rates (169, 170). A case controlled study has now suggested that this association was overstated and found no evidence of increased mortality

or complications with this ribotype when adjustments were made for other severity markers although this is not a consistent finding across all studies.

Initial therapy of CDAD is predominantly with antimicrobial therapy, particularly metronidazole or oral vancomycin in addition to discontinuing other antimicrobials where possible. (171) Vancomycin may be more effective in severe or recurrent CDAD (172). This is successful in the majority of cases, however CDAD can recur in around 25% cases with 45-65% of these patients experiencing further and sometimes multiple recurrences (173-175). CDAD can be problematic to eradicate completely and prolonged courses of vancomycin are prescribed with limited efficacy. Pulsed or tapered vancomycin therapy aims to eradicate any vegetative *C. difficile* cells that have germinated from spores since the last antibiotic exposure.

Fidixamicin is the first in a new class of macrocyclic antibiotics and has targeted activity against *C. difficile*. There is evidence that it has a less profound effect on the gut microbiota and can also limit *C. difficile* spore release. Oral fidixamicin, like oral vancomycin, has limited systemic absorption and is not metabolised via cytochrome p450 (176). Meta analysis of 2 trials of fidixamicin against vancomycin in CDAD suggested that there was a reduction in recurrent CDAD in those randomised to fidixamicin although initial cure rates were similar (8) (177). Of note, patients requiring parenteral treatment were not eligible for recruitment into trials of fidixamicin as it is administered orally only.

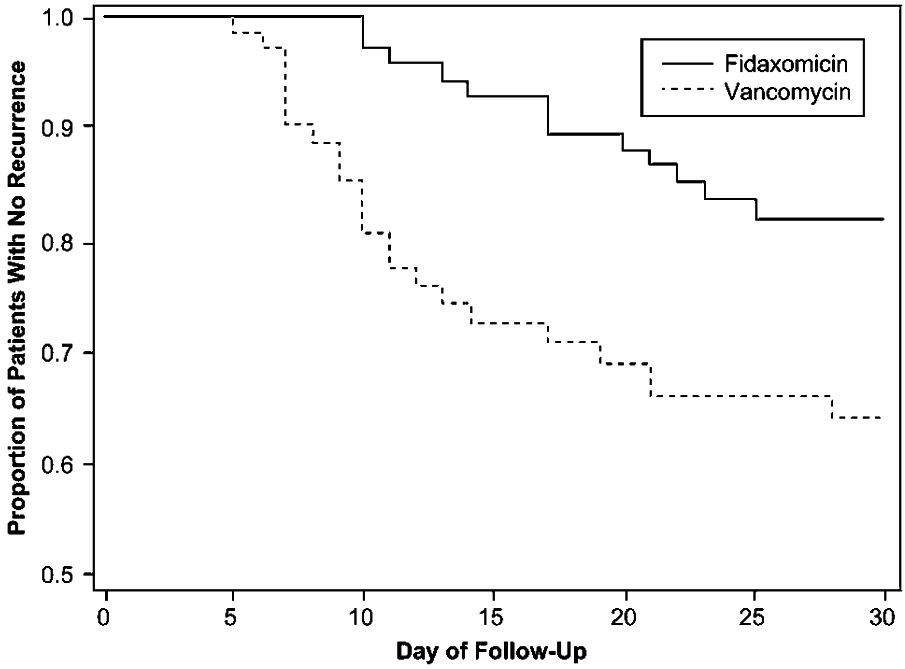
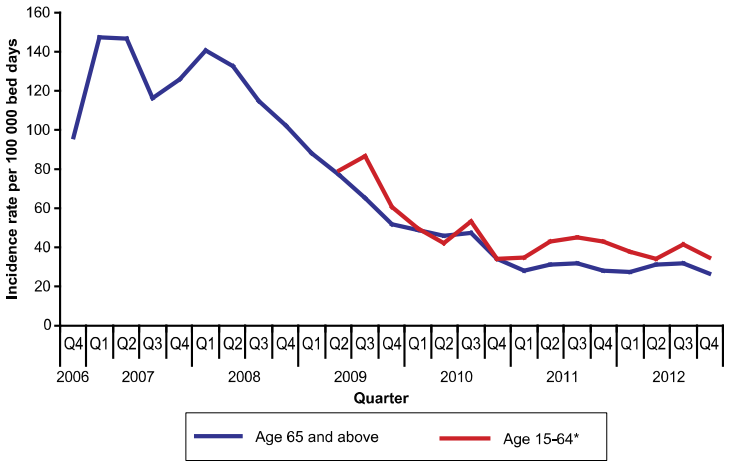


Figure 1-10 Time to recurrence by treatment group in patients with a prior episode of *Clostridium difficile* infection. Kaplan–Meier analysis of the probability of recurrence according to treatment group (per-protocol population). Day 0 is defined as the day the patient received the last dose of either fidaxomicin or vancomycin. The difference between treatment groups was statistically significant by both log rank (P = .02) and Wilcoxon (P = .01) tests (177).

CDAD in Scotland is monitored by HPS and has been falling rapidly between 2007 and 2012 from 37.4 to 29.2 per 100 000 total bed days in those aged >65 years as illustrated in Figure 1-11 below. There was also a reduction in the 027 ribotype. A range of measures are likely to account for this including increased awareness of CDAD as a cause of diarrhoea, antimicrobial stewardship and early isolation of suspected cases with strict attention to hygiene in the healthcare setting. There has been a reduction in the use of broad spectrum antibiotics in particular cephalosporins and quinolones as discussed in Chapter 2.



*surveillance in patients aged 15-64 years started April 2009

Figure 1-11 Incidence rates of CDAD in patients aged ≥65 and 15-64 years in Scotland per 100 000 bed days, October 2006 to December 2012 (Data courtesy of Health Protection Scotland (178))

Faecal transplantation is an extreme but logical method of restoring the intestinal microbiota and was first described by Eismen *et al* in 1958. Published case series have varied in the method of administration (nasogastric tube, gastroscopy, colonic delivery), donor characteristics (related vs anonymous, fresh v frozen the thawed specimen), type of suspension (sterile water, milk or saline) and indication (recurrent CDAD, refractory CDAD or both).

Case series and systematic reviews generated encouraging results, however controlled trial evidence was lacking until van Nood *et al* from the Academic Medical Centre in Amsterdam randomized 43 patients with recurrent CDAD (defined as relapse of *C. difficile* infection after at least one course of adequate antibiotic therapy) in an open labeled study to one of 3 different study groups: the infusion of donor faeces preceded by an abbreviated regimen of vancomycin and bowel lavage, a standard vancomycin regimen, or a standard vancomycin regimen with bowel lavage (179). Heavily immunosuppressed recipients, those with concurrent antibiotic use, pregnancy or critical care admission were excluded. Donors were anonymous and donor faeces specimens were screened for parasites and other faecal pathogens. Donor blood was screened for human immunodeficiency virus (HIV) in addition to other viral and bacterial infections. The primary end point was cure without relapse within 10 weeks after the initiation of therapy. Cure was defined as an absence of diarrhoea or persistent diarrhoea that could be explained by other causes with 3 consecutive negative stool samples for *C. difficile* toxin. Faecal microbiota was examined

for diversity before and after donor faeces infusion. The study was terminated early as most patients from both control arms had evidence of a relapse. There were significantly increased cure rates in those who received donor faeces compared to the vancomycin alone or vancomycin with bowel lavage groups as shown by Figure 1-12 below.

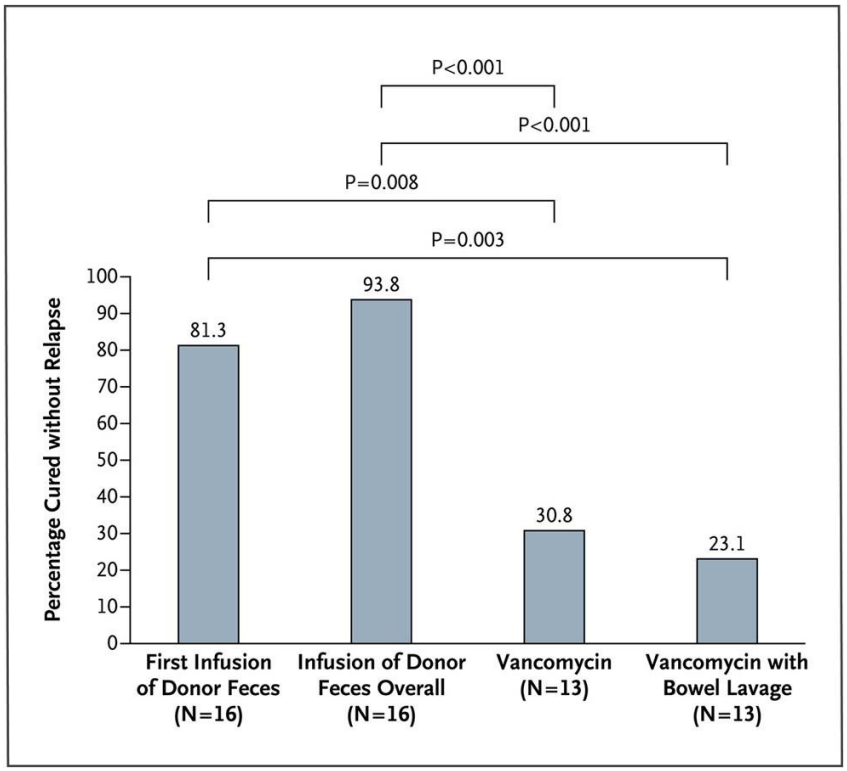


Figure 1-12 Rates of Cure without Relapse for Recurrent *Clostridium Difficile* Infection.

Shown are the proportions of patients who were cured by the infusion of donor faeces (first infusion and overall results), by standard vancomycin therapy, and by standard vancomycin therapy plus bowel lavage.

Diversity of faecal microbiota was analysed from 9 patients prior to and within 2 weeks after donor faeces infusion using Simpson's Reciprocal Index of Diversity. Diversity of the faecal microbiota was low before the infusion compared to donor faeces and compared to faecal microbiota following infusion of donor faeces. Taxa of faeces from donors and that 2 weeks following faecal transplant was indistinguishable (Figure 1-13 below).

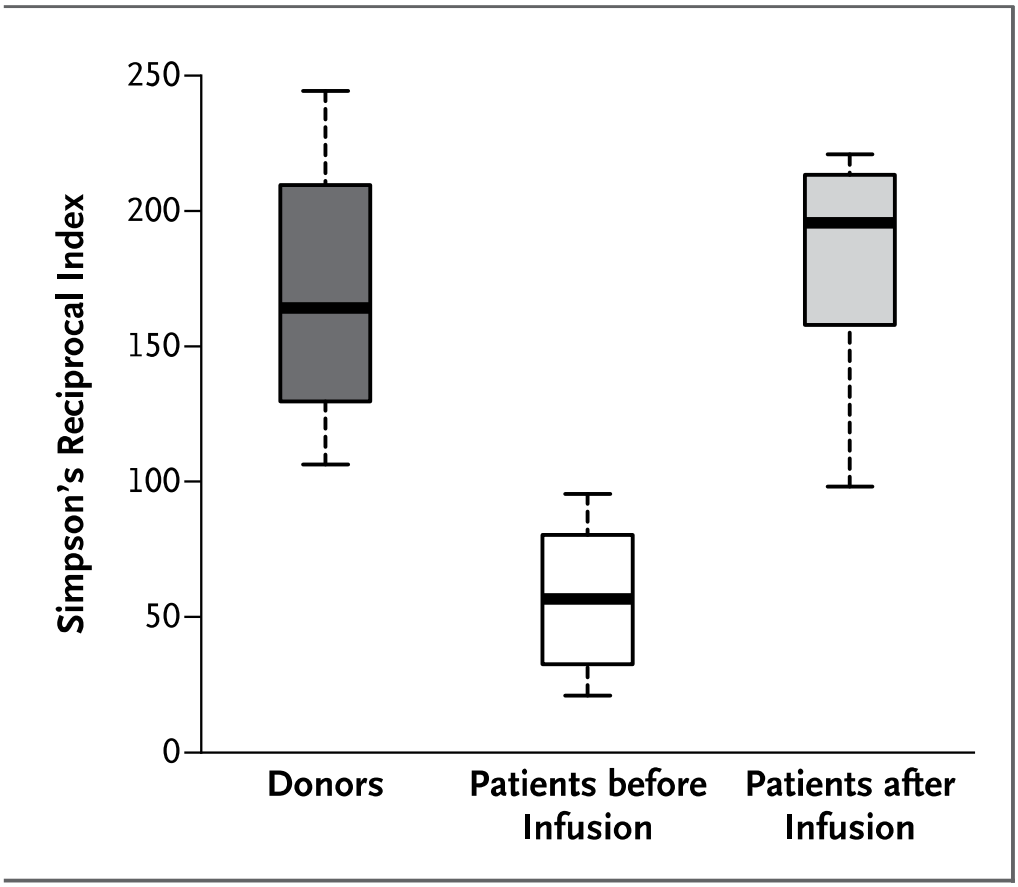


Figure 1-13 Microbiota diversity in Patients before and after Infusion of Donor Faeces, as Compared with Diversity in Healthy Donors

Microbiota diversity is expressed as Simpson's Reciprocal Index of diversity in faecal samples obtained from nine patients before and 14 days after the first infusion of donor faeces, as compared with their donors. The index ranges from 1 to 250, with higher values indicating more diversity. The box-and-whisker plots indicate interquartile ranges (boxes), medians (dark horizontal lines in the boxes), and highest and lowest values (whiskers above and below the boxes).

This well conducted study is limited by its small sample size. 35 of the 43 patients were recruited after a 2nd or greater relapse and it is known that the efficacy of antibiotic is reduced after each relapse. The nature of the intervention resulted in blinding being impossible. There is no recognized optimum antibiotic regimen for recurrent CDAD. It was reassuring that the adverse event profile was low. Research is ongoing in this field, particularly into synthetic stool (180), with a theoretical advantage of a reduction in pathogen transmission.

Probiotics and the yeast *Saccharomyces boulardii* were postulated as less invasive and less expensive methods of restoring the bowel flora and thereby preventing antibiotic associated diarrhoea, although well-designed randomised controlled studies were lacking until very recently.

1.3.5 Rationale for performing a double blind trial of probiotics in the prevention of AAD in renal inpatients

Hickson *et al* recruited 135 patients to Lactobacillus casei DN-114 001 (*L. casei imunitass*) (1.0×108 colony forming units/ml), *S. salivarius* (1.0×108 cfu/ml), and *L. bulgaricus* (1.0×107 cfu/ml) (Actimel) or placebo milkshake with striking differences in the incidence of antibiotic associated diarrhoea as illustrated by table 1-2 below (181).

Table 1-2 Total number of cases of antibiotic associated diarrhoea (including cases positive for C. difficile toxin) and proportion who were positive or negative for toxin (all patients followed up in hospital and after discharge)

	Probiotic	Control	P value*
Diarrhoea			
Yes	7 (12)	19 (34)	0.007
No	50 (88)	37 (66)	
No of patients	57†	56†	
C difficile toxin			
Positive	0	9 (17)	0.001
Negative	56 (100)	44 (83)	
No of patients	56‡	53‡	

*Fisher's exact test.
†22/135 patients lost to follow-up or withdrew.
‡4/113 patients not tested for *C difficile*.

This study has been criticized for its exclusion of high risk antibiotics and potential difficulties with blinding. Staff administering the milk drinks and monitoring for loose

stool were not blinded. Proton pump inhibitor administration was not recorded and this is a potential confounding variable.

Sampalis randomised a total of 437 patients to a probiotic BIO K+ CL1285® or placebo (182). BIO K+ CL1285® is a commercially available probiotic with a patented formula containing the *L. acidophilus* CL1285® strain of human origin, registered at the Pasteur Institute, and a *L. casei* strain. Placebo was an identical capsule containing sterile lactoserum with similar texture and taste to the probiotic. Patients were given a lower dose for the first 2 days in order to ensure tolerance of the product, followed by full dose for the remainder to the period. Both the probiotic and placebo preparations were administered within 24 hours after the first dose of antibiotic and continued once daily within ± 2 hours of the administration of the antibiotic treatment and for 5 days following the termination of antibiotic regimen. The incidence of antibiotic associated diarrhoea was reduced in the probiotic arm although this did not reach statistical significance (Table 1-3 below).

Table 1-3 Efficacy outcomes of probiotic against placebo in the prevention of antibiotic associated diarrhoea (Sampalis *et al* 2010)

	Treatment group		Odds ratio (95% CI)	*P*-value
	BIO K+ CL1285® (*N* = 216)	Placebo (*N* = 221)		
Unadjusted analysis				
Severity: mean (SD) number of days with diarrhea	0.67 (2.05)	1.19 (3.20)	NA	0.040
Incidence: (%) of patients with ≥ 1 day of diarrhea	21.8	29.4	0.667 (0.433-1.030)	0.067
Adjusted analysis*				
Severity: mean (SE) number of days with diarrhea§	0.67 (0.37)	1.19 (0.42)	NA	0.045
Incidence: (%) of patients with ≥ 1 day of diarrhea⊢	21.8	31.7	0.627 (0.405-0.971)	0.037

**Adjusted for age, duration of treatment with study product, duration of treatment with antibiotics, §Least Square Mean Estimates based on linear regression analysis, ⊢based on Multi-Variate Logistic Regression Analysis, SD – standard deviation, SE – standard error*

This study did not reach its recruitment target of 500 patients and therefore was underpowered. A Cochrane Review in 2013 concluded that more research is required in probiotics prevention of AAD and CDAD (183) and is discussed further in Chapter 5.

The PLACIDE study completed recruitment in 2012 and was published in August 2013 (184). This is the first large multicentre randomised controlled trial of probiotics against placebo and was conducted in 3 hospitals in Wales and 2 hospitals in the north of England.

They screened 17 420 patients, with 2981 patients recruited in total. 1493 were randomly assigned to the probiotic group and 1488 to the placebo group. The probiotic preparation was a lyophilised powder in a vegetarian capsule containing 6×10^{10} live bacteria: two strains of *Lactobacillus acidophilus* and two strains of *bifidobacterium*. Incidence of AAD in this study was relatively low at 10.6% with no significant difference between the probiotic and placebo groups. Interpretation of this study in the context of other positive studies is discussed further in Chapters 5 and 6.

Meta-analysis of available literature in the prevention of AAD with probiotic administration has taken this new research into account. Studies are difficult to compare having used different probiotic and yeast preparations, children and adults and a variety of clinical settings. The forest plot below (Figure 1-14) suggests a small benefit of probiotic in the prevention of AAD in older patients. Substantial statistical heterogeneity (I^2=90%) was recognised which undermines the reliability of this analysis.

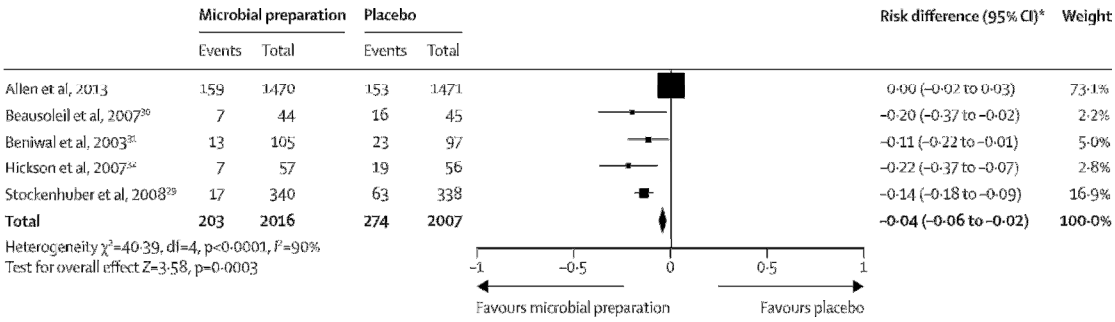


Figure 1-14 Meta-analysis of trials of lactobacilli or bifidobacteria, or both, in the prevention of antibiotic-associated diarrhoea in older inpatients (184)

Publication bias in probiotic related research has been noted on previous metanalysis with the funnel plot below (Figure 1-15) showing asymmetry with the Egger regression test (p.0.0001) and the Begg rank correlation test (p.0.0001) giving significant evidence of publication bias despite overall positive results for gastrointestinal diseases in general and antibiotic associated diarrhoea in particular.

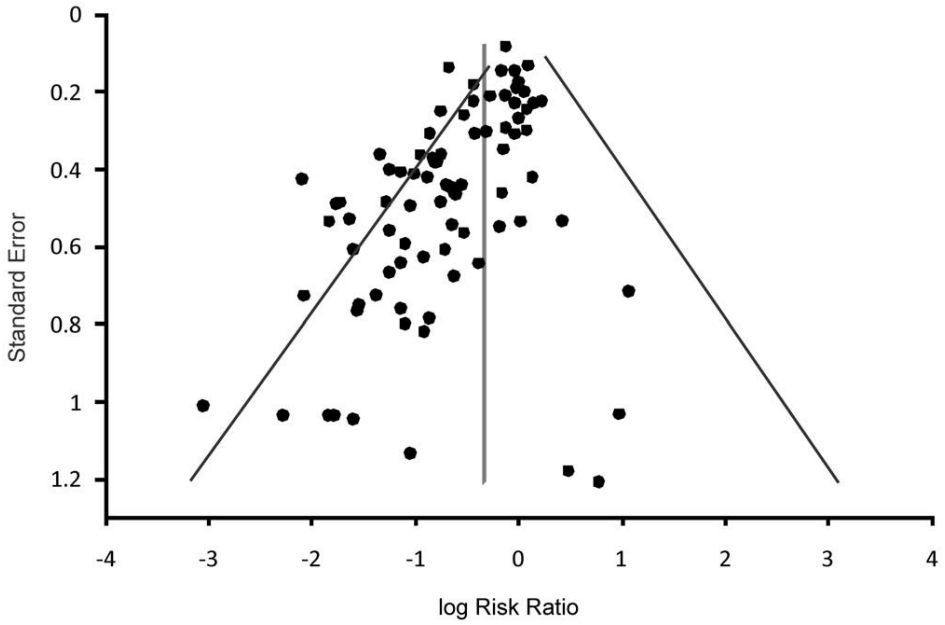


Figure 1-14 Funnel plot asymmetry used to determine publication bias. Log of the risk ratios were plotted against the standard error of the risk ratio of each study to identify asymmetry in the distribution of trials. Gaps in the funnel plot suggest potential publication bias. The synthesis estimate and the 0.01 limit are shown to distinguish asymmetry (185)

The data presented in Chapter 5 adds to the existing body evidence on the role of probiotic milk drink in the prevention of antibiotic associated diarrhoea. It is limited by its small sample size which did not reach that required by the power calculation. The study population of inpatients within a renal unit are a heterogenous group which have not been represented before in a trial of this nature.

1.4 Summary of hypotheses

This thesis examines four hypotheses in relation to infection in renal patients.

Chapter 2 hypothesises that a change in antibiotic policy resulting in increased gentamicin use would lead to a higher incidence of acute kidney injury requiring renal replacement therapy in Glasgow intensive therapy and renal units. Patients requiring emergency renal replacement therapy in the 6 months before the change in antibiotic policy and 6 months after the change in policy were compared. Gentamicin use in these patients was determined by patient casenote review and laboratory gentamicin levels.

In Chapter 3, staphylococcal bacteraemia in the Glasgow Renal Unit is examined utilising a 2 year cohort comprising renal unit inpatients and outpatients requiring regular haemodialysis. The hypothesis that patient outcomes are improved with the use of flucloxicillin compared to vancomycin in *S. aureus* bacteraemia is explored, as it's the hypothesis that *S. aureus* bacteraemia results in poorer patient outcomes compared to coagulase negative staphylococcal bacteraemia.

Chapter 4 evaluates toxin gene positivity in *S. aureus* isolates from four different patient populations with the hypothesis that *S. aureus* isolates causing disease will have a higher prevalence of toxin gene positivity. PCR testing was utilised to confirm toxin gene positivity and *spa* typing was performed with the hypothesis that there would less variety in *S. aureus* clones in haemodialysis patients.

Finally, Chapter 5 discusses a placebo controlled randomised controlled trial evaluating the effect of probiotic milk drink on the incidence of antibiotic associated diarrhoea. It hypothesises that the addition of twice daily probiotic milk drink within 48 hours of commencing antibiotics, continued for 7 days after antibiotic cessation will reduce the incidence of antibiotic associated diarrhoea.

Chapter 2: Acute kidney injury in the context of a restrictive antibiotic policy

2.1 Background

In August 2008, following a rise in *Clostridium difficile* infection, antibiotic guidelines in Greater Glasgow and Clyde health board were revised, restricting cephalosporins, co-amoxiclav and quinolones whilst promoting narrow spectrum agents and short term use of the aminoglycoside, gentamicin. As a consequence, gentamicin use doubled from approximately 20 to 40 defined daily doses / 1000 bed days as illustrated in Figure 2-1, below (p=0.002).

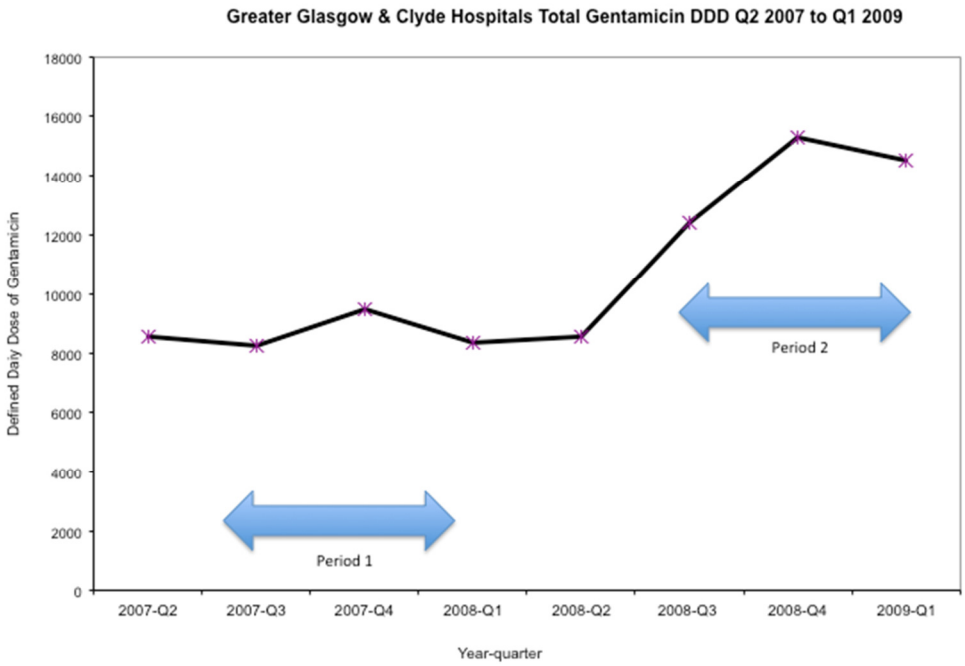


Figure 2-1 Gentamicin use by defined daily dose in Greater Glasgow and Clyde hospitals comparing 1st August 2007 until 31st January 2008 with 1st August 2008 until 31st January 2009 (p<0.05).

Acute kidney injury (AKI) affects up to 20% of hospitalised patients and is associated with increased mortality (186) (187). Gentamicin is an important cause of AKI as a result of a complex interaction of renal tubular dysfunction, tubular obstruction and reduced glomerular filtration (188).

Various mechanisms of aminoglycoside toxicity have been discussed in Chapter 1, however a clinical definition of gentamicin associated AKI has not been formally determined. Reported incidence of gentamicin associated AKI varies due to differences in study design and patient risk factors. The difficulties in defining AKI prior to the

development of validated staging criteria are discussed in Chapter 1. A rise in serum creatinine by 0.5mg/dl (44 micromol/ml) was used by Sweileh in a non critical care population with baseline normal renal function in a study designed to evaluate the nephrotoxicity of gentamicin and amikacin (189). They found that 35.6% patients prescribed gentamicin developed nephrotoxicity as defined above compared to 16.3% of those prescribed amikacin (p=0.003). Bartal *et al* defined nephrotoxicity as a rise in serum creatinine of greater than 25% from baseline or to a serum creatinine of greater than 1.4mg/dl (123micromol/l) in their study of different aminoglycoside dosing regimens and concluded that individualised dosing using an immunoassay resulted in reduced nephrotoxicity with the incidence of nephrotoxicity in the individualised dosing regimen being only 5% compared to 21% of the once daily dosing group (p=0.03). This study is limited by its small sample size with only 51 patients in total being prescribed gentamicin (190).

Cosgrove *et al* examined low dose gentamicin as adjuvant therapy in *S. aureus* endocarditis and defined an adverse renal outcome as a decrease in creatinine clearance to <50 mL/min if the baseline creatinine clearance was □50 mL/min or a decrease in creatinine clearance of □10 mL/min if the baseline creatinine clearance was <50 mL/min (101). They found that 22% of those who received gentamicin developed a deterioration in renal function compared to 8% of those who did not receive gentamicin. Several confounders were identified, in particular, three times daily dosing of gentamicin and potential nephrotoxic effects of other antibiotics such as vancomycin and high dose flucloxacillin.

Oliveira studied aminoglycoside associated nephrotoxicity in a critical care population with a baseline eGFR >30ml/min/1.73m^2 (191). They defined aminoglycoside associated nephrotoxicity as a decrease in the eGFR of 20% or more from the baseline eGFR during aminoglycoside use. They found that 58% of the 360 patients meeting inclusion criteria developed acute kidney dysfunction in association with an aminoglycoside as defined above.

In this population of patients with established acute kidney injury we have defined gentamicin associated AKI as the development of AKI requiring renal replacement therapy associated with the initiation of gentamicin between 1 and 10 days prior to the requirement for renal replacement therapy. There is no formal definition of gentamicin associated AKI and the optimal time interval for identification of gentamicin associated AKI is unclear. It

is well known that nephrotoxicity secondary to gentamicin often takes several days to manifest and therefore, given the end point of commencing RRT, the definition above would capture most patients with gentamicin associated AKI requiring RRT. This would associate gentamicin use with AKI requiring RRT although causation would be impossible to ascertain. The retrospective nature of this review necessitated an unequivocal end point such as dialysis dependence. There was no requirement for high or toxic gentamicin levels. The retrospective nature of this analysis made defining gentamicin toxicity unreliable as the time relationship between administration and therapeutic drug monitoring would not be ascertained accurately.

We investigated whether this change in antibiotic guidelines promoting the use of gentamicin resulted in any change in the incidence of, or mortality associated with, AKI with a secondary end point of residual renal impairment.

2.2 Methods

2.2.1 Data collection

A retrospective audit of all patients requiring emergency renal replacement therapy for AKI within all Greater Glasgow and Clyde Health Board hospitals over two time periods was performed. The first period, (Period 1), before the change in antibiotic guidelines from 01/08/2007-31/01/2008, and the second, (Period 2), after the change in antibiotic guidelines 01/08/2008-31/01/2009. The audit periods were separated by 6 months allowing a run in period for the change in antibiotic guidelines and to minimise seasonal bias.

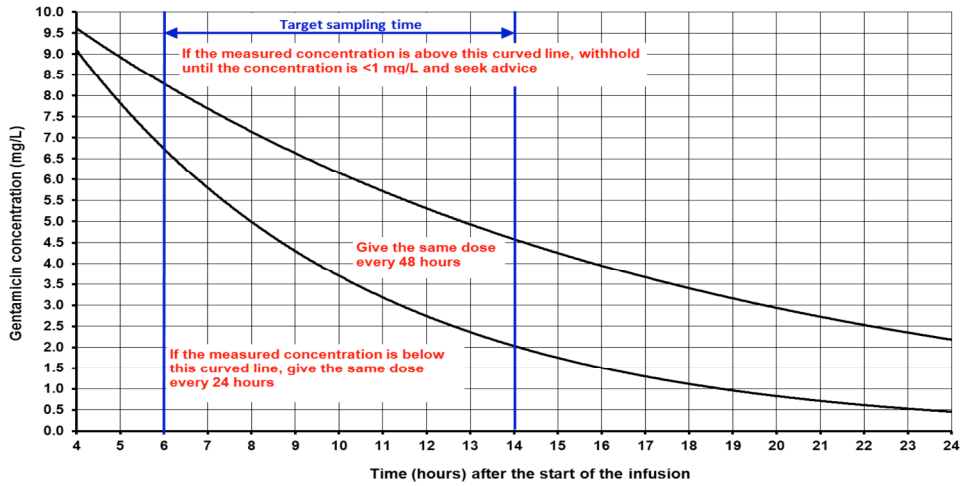
All general intensive therapy units (ITUs) and renal units in GGC were included. Patients undergoing renal replacement therapy in an intensive therapy unit were identified using the "Wardwatcher" software used by all intensive therapy units in Greater Glasgow and Clyde (GGC). This records prospectively collected data of all ITU inpatient interventions including use of RRT. Date of first dialysis session in an inpatient renal unit, or outlying area such as a coronary care unit, during the study period was initially identified using the renal unit electronic patient record then confirmed where possible using paper case notes. Patients commencing dialysis as a consequence of progressive chronic kidney disease

(CKD) were identified by a pre-existing eGFR of <15ml/min/1.73m^2 prior to this acute presentation and excluded from the analysis.

Paper case notes, electronic clinical portal, electronic renal database and biochemistry results were reviewed for each patient to assess co-morbidities, likely causes of AKI, dates of gentamicin use, duration of renal replacement therapy, mortality and extent of renal recovery. Patients with primary glomerulonephritis were included in this population. Vascular disease was defined as the presence of ischaemic heart disease, cerebrovascular disease or peripheral vascular disease affecting large of small vessels. Immunosuppression includes high dose corticosteroids, in addition to antiproliferatives, and other drugs affecting the immune response.

Patients with pre-existing Stage 5 chronic kidney disease (including those patients with functioning renal transplants but eGFR<15ml/min/1.73m^2) were excluded. Patients already requiring dialysis, patients transferred from hospitals outside GGC Health Board and patients developing AKI following cardiac surgery were all excluded as antimicrobial guidelines in these patients differed from those in NHS GGC and the population of interest was those with AKI in general, and gentamicin-associated AKI in particular.

In time period 1, recommended gentamicin dosing was based on a 24 or 48 hourly dosing system (approximately 5mg/kg) utilising body weight with creatinine clearance estimated using the Cockcroft-Gault calculation. After auditing compliance with these guidelines, and estimating pharmacokinetic profiles, the gentamicin dosing regimen was simplified prior to Period 2. The dose range was 180mg every 48hours to 400mg every 24 hours, with a 2.5mg/kg (maximum dose 180 mg) recommended for patients with a CrCl<20ml/min. This ensured either 24 or 48 hourly dosing and uniformity of prescribing guidance across all hospitals. An intranet-based gentamicin dosage calculator, dosing chart (according to Cockroft and Gault creatinine clearance calculation) and plot to guide sampling time, target trough levels and subsequent dosage (Figure 2-2, illustrated below) was introduced to further simplify dosing.



MONITORING OF GENTAMICIN CONCENTRATIONS	
1.	Take a blood sample 6 – 14 hours after the start of the first infusion.
2.	Record the exact times of the dose and sample on the recording chart.
3.	Plot the concentration measurement on the graph.
4.	The plot will indicate one of 3 options: (1) Continue present dosage regimen, (2) adjust dosage interval or (3) withhold and resample after 24 hours.
5.	Seek advice if you are unsure how to interpret the result.
6.	Monitor creatinine concentrations daily.
7.	Take a further sample 6-14 hours post dose, at least every 2 days.
8.	If the concentration is unexpectedly high, or if renal function alters, daily sampling may be necessary.

Figure 2-2 Gentamicin dose adjustment plot

Demographic, comorbidity, gentamicin usage and outcome data from patients with AKI requiring RRT from Period 1 and Period 2 were compared. Following this, patients identified as having gentamicin-associated AKI from either time period were compared to the rest of the cohort.

2.2.2 Statistical analyses

The Mann Whitney U test was used to compare data that were not normally distributed. The binomial comparison of 2 distributions and binary logistic regression analysis were utilized where appropriate using Minitab 13.1 software. A univariate binary logistic regression analysis, to identify factors associated with a higher risk of death, was performed using the entire cohort. Those with $p<0.15$ were entered into multivariate analysis using a backwards selection model.

2.3 Results

No change in the incidence of AKI requiring RRT was identified. 191 patients who received emergency renal replacement therapy were identified during Period 1 (01/08/2007-31/01/2008) and 184 identified during Period 2 (01/08/2008-31/01/2009).

2.3.1 AKI: Comparison between Period 1 and Period 2

There was no significant difference in patient age, length of hospital stay, incidence of sepsis or mortality comparing the 2 periods. 43% of patients in both populations received gentamicin at any time during their admission. There were no significant differences in patient comorbidity comparing the patients identified in the 2 periods (Table 2-1).

Table 2-2 compares patient outcomes and gentamicin use in Period 1 compared with Period 2. There was no significant difference in mortality or length of patient admission between the 2 groups. Duration of gentamicin use was significantly more prolonged during Period 1 before the change in antibiotic guidelines ($p=0.046$) although this was not maintained when gentamicin use was censored for death ($p=0.097$). This may represent increased antimicrobial stewardship and increased awareness of gentamicin-associated AKI during Period 2. There was no significant difference in mortality or in the incidence of patients alive but with a deterioration in baseline serum creatinine comparing the 2 periods studied.

Table 2-1 Baseline characteristics and comorbidities of patients with AKI requiring RRT comparing Period 1 (before change in antibiotic guidelines) and Period 2 (following change in antibiotic guidelines).

Data are expressed as value (%) or median (interquartile range) as appropriate

	Period 1 01/08/2007-31/01/2008 (n=191)	Period 2 01/08/2008-31/01/2009 (n=183)	p value
Age (years)	66 (54, 74)	63 (51, 74)	0.393
Male	115 (60.2)	110 (60.1)	0.984
AKI risk factors			
ITU admission	138 (72.25)	129 (70.49)	0.707
Renal unit admission only	50 (26.2)	51 (27.9)	0.713
Sepsis	145 (75.92)	129 (70.49)	0.236
Hypotension	102 (53.40)	89 (48.63)	0.356
Volume depletion	129 (67.54)	114 (69.30)	0.288
Urinary tract obstruction	6 (3.14)	6 (3.28)	0.940
Surgery	41 (21.47)	47 (25.68)	0.337
Iodinated contrast	15 (7.85)	20 (10.93)	0.308
Comorbidities			
Diabetes mellitus	36 (18.85)	38 (20.77)	0.642
Vascular disease	61 (31.94)	58 (31.69)	0.960
Acute myocardial infarction	12 (6.28)	16 (8.74)	0.367
Medications			
Gentamicin at any time during admission	76 (39.79)	77 (42.08)	0.653
ACE-I*/ARB **	52 (27.23)	57 (31.15)	0.404
NSAID[§]	14 (7.33)	9 (4.92)	0.329
Diuretic	51 (26.70)	50 (27.32)	0.892
Immunosuppression	17 (8.90)	15 (8.20)	0.808

*** Angiotensin-converting enzyme inhibitor**

**** Angiotensin 2 receptor blocker**

[§] Non steroidal anti inflammatory drugs

Table 2-2 Comparison of outcomes and gentamicin use between Period 1 (before change in antibiotic guidelines) and Period 2 (after change in antibiotic guidelines). Data are expressed as value (%) or median (interquartile range) as appropriate

	Period 1 01/08/2007- 31/01/2008 n=191 total n= 89 alive at discharge	**Period 2 01/08/2008- 31/01/2009 n= 183 total n= 99 alive at discharge**	**p value**
Length of stay in days: all	20 (8, 38.5)	20 (9, 34.5)	0.598
Length of stay in days: those who survived to discharge	28 (18.25, 50)	27 (17, 43)	0.588
Duration of gentamicin in days: all	N= 61 4.5 (0, 9)	N=73 3(1,7)	**0.046**
Duration of gentamicin in days : those who survived to discharge	N=27 5.5 (2, 9.75)	N=29 2 (1,7.5)	0.097
Alive: no renal recovery	5/89	5/99	0.863
Alive with >20% deterioration in baseline serum creatinine at hospital discharge	13/89 (14.6)	21/99 (21.21)	0.235
In Patient Mortality	102/191 (53.4)	84/183 (45.9)	0.146
Mortality: 1 year	112/191 (58.6)	101/183 (55.2)	0.501

2.3.2 Comparison of gentamicin-associated AKI to the remainder of the cohort

Overall, 40.5% (61 patients) of patients who received gentamicin were classified as gentamicin-associated AKI, having started gentamicin therapy between 1 and 10 days prior to requiring renal replacement therapy. 27 of these patients (44.3%) were admitted during Period 1 and 34 patients (55.7%) during Period 2.

The gentamicin-associated AKI group was more likely to have undergone surgery and had a higher number of cumulative AKI risk factors defined as the number of risk factors,

comorbidities and predisposing medications as illustrated in Table 2-3. The gentamicin associated AKI group had a relatively short duration of gentamicin therapy with a median of 2 days. In those who survived to discharge from hospital, there was no significant difference in the duration of gentamicin therapy (p=0.44). Patients with gentamicin associated AKI had a more prolonged hospital stay (p=0.16) although clearly the increased incidence of ITU admission is a major confounder here.

Despite the higher proportion of ITU admission, greater number of risk factors and longer duration of stay, there was no evidence of increased inpatient or 1 year mortality in the gentamicin associated AKI group. The gentamicin associated AKI group and all other AKI had 1 year mortality of 57.4% and 55.4% respectively. This is in keeping with data for those requiring acute renal replacement therapy in previous research (5, 192). Data illustrated in Tables 2-3 and 2-4 underwent Bonferroni correction for multiple comparisons and therefore the P value for statistical significance was adjusted from <0.05 to <0.003 in Table 2-3 and corrected to <0.005 in Table 2-4.

Table 2-3 Baseline characteristics and AKI risk factors comparing those with gentamicin associated AKI to the remainder of the cohort.

Data are expressed as value (%) or median (interquartile range) as appropriate. Statistical significance was defined at p<0.002 after Bonferroni correction for multiple comparisons

	Gentamicin-associated AKI n=60	All other AKI n=315	p value
Age (years)	65.0 (57.0-71.5)	65.0 (51.0-74.0)	0.93
Sex (male %)	37 (60)	186 (60)	0.96
AKI risk factors			
ITU admission	51 (83.6)	220 (70.1)	0.004
Renal unit admission only	9 (14.8)	91 (30.0)	0.008
Sepsis	58 (95.1)	220 (70.1)	**0.000**
Hypotension	36 (59.0)	156 (49.7)	0.33
Volume depletion	44 (72.1)	200 (63.7)	0.53
Urinary tract obstruction	1 (1.6)	11 (3.5)	0.80
Surgery	27 (44.3)	62 (19.7)	**0.000**
Iodinated contrast	10 (16.4)	26 (8.3)	0.39
Comorbidities			
Diabetes mellitus	11 (18.0)	63 (20.1)	0.59
Vascular disease	20 (32.8)	99 (31.5)	1.00
Acute myocardial infarction	6 (9.8)	22 (7.0)	0.75
Medications			
ACE-I/ARB	20 (32.8)	89 (28.3)	0.77
NSAID	4 (6.6)	19 (6.1)	0.99
Diuretic	17 (27.9)	84 (26.8)	0.93
Immunosuppression	10 (16.4)	23 (7.3)	0.063
Number of AKI risk factors	7.0 (5,8)	5.0 (3,6)	**0.000**

Table 2-4 AKI outcomes comparing those with gentamicin associated AKI to the remainder of the cohort.

Data are expressed as value (%) or median (interquartile range) as appropriate. Statistical significance was defined at p<0.005 after Bonferroni correction for multiple comparisons

	Gentamicin-associated AKI n=60	All other AKI n=315	p value
Length of stay in days: all	24.0 (14.0-42.5)	19.0 (8.0-34.8)	0.016
Length of stay in days: those who survived to discharge	37.0 (26-74)	25 .0 (16-44.8)	**0.003**
Duration of gentamicin use in days: all	2.0 (1.0-7.0)	4.0 (1.0-9.0)	0.048
Duration of gentamicin use in days: those who survived to discharge	5.0 (2.3-10.0)	5.0 (2.5-9.5)	0.44
Survived with new residual renal impairment	5 (8.20)	30 (9.5)	0.762
Survived with no renal recovery	0	6 (1.9)	0.013
Mortality (inpatient)	32 (52.5)	154 (49.0)	0.72
Mortality (1 year)	35 (57.4)	174 (55.4)	0.81

2.3.3 Outcomes and regression analyses using the entire cohort

327 of 374 patients (87.4%) had information on baseline renal function. Renal outcomes are summarised in Figure 2-3.

181 (48.3%) of these patients survived until discharge from hospital and 153 (46.8%) were alive at 1 year. Of those 181 patients who survived to discharge, 115 patients (63.5%) had normal renal function at presentation, 38 patients (20.1%) had stage 3 CKD and 15 patients (8.2%) had stage 4 CKD. 13 of the patients who survived to discharge had no recent biochemistry results therefore their baseline level of renal function was unknown. As

would be expected, increasing age was associated with pre-existing CKD in patients surviving to hospital discharge (OR 2.25 for every 10 year increase in age, 95% confidence intervals 1.63, 3.12, p=0.000) and in the entire cohort (OR 2.31 for every 10 year increase in age, 95% confidence intervals 1.82, 2.93, p=0.000).

At the time of discharge, 31 of 181 (18.1%) patients had a decline in renal function compared to baseline including 10 patients (5.8%) remaining dialysis dependent. 17 of these patients (54.8%) had pre-existing CKD with 11 patients (35.4%) having stage 3 CKD and 6 patients (19.4%) stage 4 CKD. 7 patients of the 53 with known baseline CKD who survived to hospital discharge became dialysis dependent (11.3%). 3 of these patients had pre-existing stage 3 CKD and the remainder had stage 4 CKD.

47 patients from the total population had no pre-existing biochemistry. 13 of these patients survived to hospital discharge. 6 patients in this group did not recover to an eGFR of >60ml/min/1.73m^2 and 6 patients were dialysis dependent. It is likely that a proportion of patients had unrecognised CKD prior to presentation with an acute illness.

To determine factors associated with a deterioration in renal function or dialysis dependence after the episode of AKI a univariate analysis was performed on the entire cohort. Gentamicin administration had no impact on renal outcome. A higher premorbid serum creatinine (p=0.002), increasing age, location of RRT (renal unit) and death at 1 year were associated with an increased risk of deterioration in renal function after the episode of AKI (Table 2-4). Multivariate analysis confirmed that poorer baseline renal function was highly associated with residual renal impairment. Patients were also more likely to have residual renal impairment if they received RRT in a renal unit only. There were 7 patients with acute glomerulonephritis in this cohort. One of these patients died during the follow up period, 1 patient fully recovered renal function, 2 required long term dialysis and 3 had residual renal impairment at hospital discharge and at 1 year with 1 of these patients requiring RRT at some point during their admission.

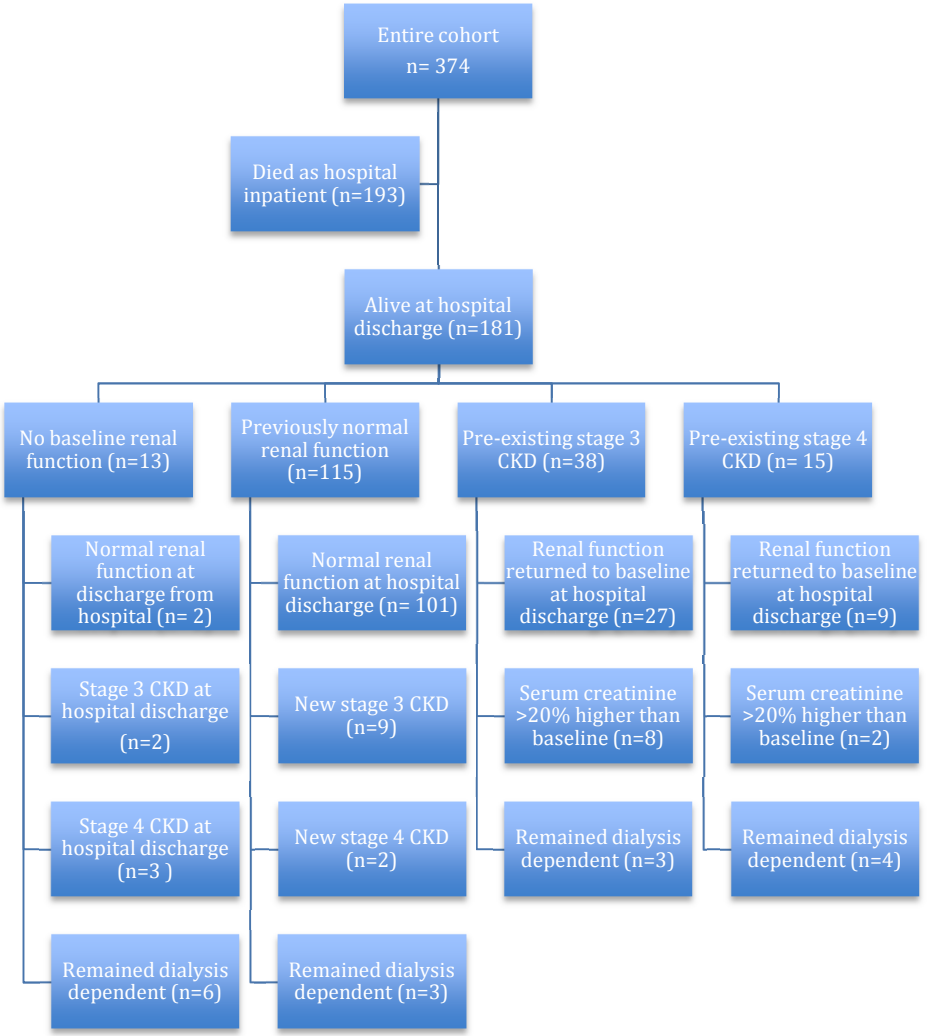


Figure 2-1 Hierarchy plot of renal outcomes in surviving patients

Table 2-5 Univariate and multivariate binomial regression analysis of factors associated with residual renal impairment at discharge from hospital. Multivariate analysis was performed using a backwards selection model.

Variable	Univariate analysis Odds Ratio	95% CI[a]	P value	Multivariate analysis Odds Ratio	95% CI	P value
Age[b]	1.54	1.15, 2.05	0.002			
ITU admission	0.14	0.06, 0.33	0.000	0.18	0.08, 0.44	0.000
Renal Unit admission only	6.06	2.67, 13.77	0.000	4.66	1.98, 10.99	0.000
Baseline serum creatinine (micromol/l)[c]	1.13	1.04, 1.02	0.002	1.11	1.01, 1.21	0.000
Gentamicin use at any time	0.27	0.10, 0.68	0.003			
Death at 1 year	4.40	1.63, 11.88	0.005			

[a]Confidence Interval

[b]Per 10 year increase in age

[c]Per 10 micromol/l rise in serum creatinine

There was no trend towards more prolonged hospital admission according to age in those surviving to discharge (Pearson correlation coefficient -0.11, P value 0.123). This did not change when the renal unit population only was studied (Pearson correlation coefficient 0.187, P value 0.11).

To determine factors associated with inpatient mortality, univariate binary logistic regression analysis was performed on the entire cohort. ITU admission, increasing age, sepsis, immunosuppression and gentamicin use at any time were associated with an increased risk of in hospital mortality. A prior diagnosis of diabetes mellitus and ACE-inhibitor or ARB use were associated with decreased risk of in hospital mortality. An increased burden of AKI risk factors was not associated with increased mortality (Odds ratio 1.08, 95% confidence intervals 0.98, 1.20).

Multivariate analysis was subsequently performed using a backwards selection model. ITU admission and increasing age were associated with increased risk of death whereas angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker use prior to renal replacement therapy was associated with decreased risk of death. There was no association with increased in hospital mortality with gentamicin exposure prior to renal replacement therapy (gentamicin-associated AKI) or with gentamicin exposure at any time. Univariate and multivariate analyses are illustrated in Table 2-6.

Table 2-6 Binary logistic regression analysis of factors associated with in-hospital mortality. Multivariate analysis was performed using a backwards selection model.

Variable	Univariate analysis Odds Ratio	95% CI[a]	P value	Multivariate analysis Odds Ratio	95% CI	P value
ITU admission	3.81	2.32, 6.27	0.000	4.87	2.65, 8.23	0.000
Age	1.16[b]	1.01, 1.32	0.03	1.44 [b]	1.22, 1.70	0.000
Sepsis	1.53	0.90, 2.61	0.116			ns
Diabetes mellitus	0.46	0.27, 0.79	0.004			ns
Immunosuppression	2.28	1.07, 4.86	0.028			ns
Gentamicin use at any time	1.60	1.05, 2.43	0.028			ns
Prior ACE I/ARB[c]	0.49	0.31, 0.78	0.002	0.45	0.24, 0.82	0.01

[a]Confidence Interval

[b]Per 10 year increase in age

[c]Angiotensin 2 receptor blocke

2.4 Discussion

This large dataset from a single health board has investigated patients requiring RRT for AKI before and after a change in antimicrobial policy that resulted in a doubling of gentamicin use. We have found no evidence of increased requirement for RRT for AKI, no change in mortality associated with requiring RRT for AKI and no change in renal outcomes. Patients who received gentamicin during Period 1 had a more prolonged overall duration of therapy, however this may reflect specific advice to limit gentamicin therapy duration in Period 2 and increased antibiotic stewardship.

Patients with gentamicin-associated AKI were defined as those receiving gentamicin between 1 and 10 days prior to RRT requirement. There was no evidence of increased mortality in this patient group compared to the remainder of the cohort. This was despite a higher burden of AKI risk factors in this patient group. It is notable that 94 of 314 (29.9%) patients in the non-gentamicin associated AKI group also received gentamicin. Gentamicin use at any time was associated with increased risk of death on univariate analysis but not multivariate analysis, and may simply be a surrogate marker for sepsis, especially in the ITU setting.

Gentamicin prescribing increased as a consequence of restricted prescribing of co-amoxiclav, cephalosporins and quinolones due to their association with C. difficile associated disease (CDAD). CDAD incidence has fallen as a result of this and other interventions as discussed in Chapters 1 and 5. Antimicrobial prescribing is measured by defined daily dose (DDD). There was a significant increase in gentamicin prescribing between the 2 study periods ($p=0.002$) but no corresponding increase in RRT requirement or mortality giving a favourable risk: benefit ratio despite concerns at implementation of the change in guidelines of increased gentamicin associated AKI by virtue of increased gentamicin prescribing.

Multivariate analysis was consistent with previous data showing that increasing age and requiring RRT in an ITU environment are associated with an increased risk of mortality. The suggestion that a diagnosis of diabetes could be a positive prognostic marker should be viewed with caution. This has not been observed in other research and it is important to recognise that the population studied are a highly selected group who were felt appropriate for critical care admission or emergency renal replacement therapy.

The presence of proteinuria has been associated with increased risk of requiring RRT post cardiac surgery, although there was no evidence of diabetes mellitus being implicated in a large observational study (193). This is contrasted with retrospective data from nearly 450 000 patients undergoing coronary artery bypass grafting suggesting that diabetes, treated with either oral agents or insulin, increased the risk of postoperative dialysis with Odds Ratios of 1.42 and 2.17 respectively (194).

Prior use of an ACE inhibitor or angiotensin 2 receptor blocker also appeared to be associated with decreased mortality on multivariate analysis. This has not been observed in previous literature and should be explored further. There may be an argument that patients with AKI with ACE inhibitor or ARB as an exacerbating factor are less unwell at presentation, however in this cohort, potential confounders such as concomitant sepsis and ITU admission were included in the multivariate analysis. These agents are highly reversible contributing factors to AKI that have been associated with increased mortality in a cardiac surgery population in retrospective series (195). There may also be an increased risk of AKI when ACE inhibition is associated with iodinated contrast administration although prospective research is lacking and existing data is contradictory (196, 197).

The size of the dataset has allowed further analysis into risk factors associated with hard outcomes such as residual renal impairment. 20.3% patients were discharged from hospital with residual renal impairment and 5.3% became dialysis dependent. This is a lower proportion than that observed in other critical care populations (198), and in a meta-analysis of AKI in adults, which also recognised the heterogenous nature of available research (199). They found that increased age was associated with non recovery of AKI requiring RRT, with 31.3% of surviving elderly patients not recovering kidney function compared with 26% of younger patients (pooled relative risk, 1.28, 95% confidence interval, 1.06 to 1.55; $P < 0.05$).

Patients who received RRT in a renal unit only were more likely to have residual renal impairment at discharge from hospital. This may relate to other patient characteristics not captured by this analysis including the underlying cause of renal impairment and the decreased overall mortality in this population.

These data are limited by the definition of AKI as dependence on renal replacement therapy and its retrospective nature. It is well recognised that very small rises in serum creatinine are associated with significant morbidity and mortality and therefore further

research into gentamicin and AKI not requiring dialysis is required. Prospective data collection would help to capture a full dataset ensuring that, for example, single doses of gentamicin not followed up by levels are recorded. Ongoing surveillance of outcomes in gram negative bacteraemia in NHS GGC with research in press suggests that there has been a fall in mortality in those with hospital associated infection with associated reduction in resistant organisms since the change in antibiotic guidelines. As seen in these data, there was no change in the incidence of acute RRT.

A very large sample size would be required to identify an increase in AKI requiring RRT as a result of gentamicin use. If we assume that 10% of patients admitted to an ITU require RRT, in order to identify a 20% increase in RRT requirement with 80% power and type 1 error rate 0.05, we would require a minimum sample size of 7700 patients. Such a prospective study would be difficult to recruit, an alternative antibiotic to gentamicin be required for ethical approval and blinding would be impossible due to the requirement for drug level monitoring. Therefore such a trial would be unlikely to proceed to a recruitment stage. This raises the potential for the current study to be a false negative result as it is underpowered.

2.5 Conclusion

In summary, in this retrospective cohort, we have found no evidence of an increase in gentamicin-associated AKI requiring renal replacement therapy despite doubling of gentamicin use in the hospital population. Our concern that increased gentamicin use may result in a significant increase in AKI requiring emergency renal replacement therapy was unfounded**.**

Continued vigilance is required to ensure that gentamicin is used safely and appropriately, particularly through careful therapeutic drug monitoring, avoidance of other co-administered nephrotoxic agents and restriction in duration of therapy.

2.5 Suggestions for further research

Further research in this field should focus on the prevention of gentamicin-associated AKI by adjusting gentamicin dosing regimens in order to adequately treat sepsis while reducing the potential for toxic levels with limitation of gentamicin course duration.

Further prospective research into the early recognition of AKI and addressing its precipitants with the aim of improving outcomes is required. Methods to facilitate the detection of early acute kidney injury using automatic electronic alerts from the biochemistry laboratory are already underway with promising results (34). A need for a more robust and consistent assessment of patients with AKI was highlighted by the NCEPOD Report(11) and prospective research may include a bundle of measures similar to that recommended in the assessment of the patient with possible sepsis (200). These measures may help to increase awareness of gentamicin-associated AKI in this era of restricted antimicrobial prescribing.

Global surveillance by the World Health Organisation has identified very high rates of resistance in common bacteria including S. aureus (201). A post antibiotic era during the 21st century is becoming a very real possibility with significant associated health and economic implications. Alternative approaches, such as improved and more rapid diagnostics and non-antimicrobial approaches to the prevention and treatment of infection may help to delay or avert this potential disaster.

Chapter 3: A Retrospective Study of Staphylococcal Bacteraemia in a Renal Unit.

3.1. Background

Staphylococcus spp. bacteraemia is a significant cause of morbidity and mortality in renal failure populations. Metastatic infective complications are relatively common e.g. endocarditis can occur in over 10% of episodes of *S. aureus* bacteraemia (202). Endocarditis associated with coagulase negative staphylococcal spp is much less common, developing in fewer than 3% cases (203).

HD access is often implicated as the route of inoculation, especially where central venous catheters have been used. It is well recognised that regular HD patients with tunnelled and non tunnelled central venous catheters have an increased incidence of bacteraemia and increased risk of mortality (99). Antimicrobial line locks may reduce the risk of catheter-associated bacteraemia, however there are concerns regarding the promotion or selection of resistant organisms (204). Cannulation of arteriovenous fistulae via the buttonhole method is also associated with an increased incidence of bacteraemia despite an overall reduction in radiological and surgical interventions required to maintain a functional fistula (205) (206).

Sepsis is reported to account for 18% of all cause mortality in prevalent dialysis patients (207). Renal units account for nearly 5% of all of the episodes of methicillin sensitive *Staphylococcus aureus* (MSSA) bacteraemia reported to Health Protection Scotland (98). *Staphylococcus* spp. are the predominant organisms causing bacteraemia in a HD population as discussed in Chapter 1.

Previous research has suggested that patients with established renal failure and MSSA bacteraemia have higher hospitalisation and mortality rates when treated with vancomycin instead of the beta lactam antibiotic, cefazolin (208). However there is no consensus on the optimal antibiotic duration or antibiotic choice in treatment of MSSA bacteraemia in the HD population as discussed in Chapter 1. The majority of coagulase negative staphylococci causing disease are *S. epidermidis*, however no species-specific information was available for the purposes of this research.

This study was performed to:
a) determine the effect of *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. bacteraemia on mortality, metastatic infection and hospitalisation rates in patients with established renal failure.
b) compare different antimicrobial regimens in the treatment of MSSA bacteraemia.
c) evaluate the epidemiology of the *Staphylococcus aureus* species with respect to spa gene typing and diversity of the species involved.

3.2 Methods

3.2.1 Data collection

All positive blood cultures of *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. from patients who were receiving outpatient HD therapy or were a renal inpatient under care of the Glasgow Renal and Transplant unit between 01/01/2010 and 31/12/2011 were obtained from the microbiology laboratories in Greater Glasgow and Clyde and Forth Valley health boards. Details of HD access type at the time of bacteraemia, hospitalisation, evidence of metastatic infection and mortality were recorded. Where available, data on antibiotic therapy were recorded from the Glasgow Renal and Transplant Unit Electronic Patient Record.

In this cohort, the definition of bacteraemia was entirely microbiological with no requirement for evidence of localised infection, metastatic infection or a systemic inflammatory response. Positive blood cultures for the same organism within 14 days were considered the same episode of bacteraemia.

All demographic and admission data was obtained from the Glasgow Renal and Transplant Unit Electronic Patient Record and NHS Greater Glasgow and Clyde Clinical Portal. These collate admission data, blood test results, radiology reports and records of procedures performed for all patients under the care of the Glasgow Renal and Transplant Unit, Western Infirmary, Glasgow.

Spa gene typing of all *S. aureus* positive blood cultures was performed as standard in the Scottish MRSA Reference Laboratory (209). PCR amplification was performed in house using Ridom primers prior to *Spa* testing by GATAC Biotech (Germany) (210).

3.2.2 Statistical analyses

The Mann Whitney U test, binomial comparison of 2 distributions and binary logistic regression analysis were utilized where appropriate using Minitab 13.1 software. The continuous data were not normally distributed by the Anderson-Darling test for normality.

3.3 Results

3.3.1 Staphylococcus spp. bacteraemias

248 renal inpatients with 362 episodes of staphylococcal bacteraemia were identified during the two year study period.

- Eleven (3.0%) episodes were in renal transplant recipients
- Forty-seven (13.0%) were in HD patients dialysed via arteriovenous fistulae
- Two hundred and twenty-five (62.2%) were in HD patients dialysed via tunnelled central venous catheters
- Fifty-one (14.1%) were in HD patients who dialysed via non tunnelled central venous catheters
- Twenty-eight (7.7%) were in renal inpatients not receiving RRT and with no indwelling central vascular access

Sixty-five of the 248 (26.2%) patients died within 6 months of a first episode of staphylococcal bacteraemia. Renal transplant recipients were less likely to die in the 6 months after a first episode of staphylococcal bacteraemia ($p = 0.04$) compared to patients requiring regular HD.

Eighteen of the 248 (7.3%) patients had evidence of metastatic infection.

- Eleven patients (4.4%) had endocarditis
- Three patients (1.2%) had discitis
- Two patients (0.8%) had osteomyelitis
- Two patients (0.8%) had mycotic abscess formation

There were an average of 1.32 admissions per 6 months before a first episode of staphylococcal bacteraemia and 1.67 admissions per 6 months after the first episode of staphylococcal bacteraemia ($p = 0.01$). Patients who developed bacteraemia had an average of 9.59 inpatient days per 6 months before the first episode of staphylococcal bacteraemia and 14.59 inpatient days per 6 months after the first episode of staphylococcal bacteraemia ($p = 0.00$).

There was no significant difference between the number of vascular access procedures in the 6 months before (1.02 procedures) or after (1.10 procedures) developing a first episode of staphylococcal bacteraemia.

3.3.2 Staphylococcus aureus bacteraemia (MSSA and MRSA combined)

There were 84 renal inpatients with 102 *S. aureus* bacteraemias (SAB) (including 16 MRSA bacteraemias).

- Four (3.9%) SAB were in renal transplant recipients
- Nineteen (18.6%) SAB were in HD patients dialysed via arteriovenous fistulae (AVF)
- Sixty-one (59.8%) SAB were in HD patients dialysed via tunnelled central venous catheters (TCVC)
- Fourteen (13.7%) SAB were in HD patients dialysed via non tunnelled central venous catheters (NTCVC)
- Four (3.9%) SAB were in renal patients who were not undergoing HD and were not renal transplant recipients

Twenty-three (27.4%) of the patients died within 6 months after first developing *S. aureus* bacteraemia. None of these patients were renal transplant recipients ($p=0.04$). Seven (14.6%) of the patients had metastatic infection and only 1 of these patients dialysed via an AVF.

There were an average of 1.41 admissions per 6 months before and 2.08 admissions per 6 months after first developing SAB ($p < 0.01$). Patients had an average of 11.26 inpatient

days per 6 months before and 15.79 inpatient days per 6 months after developing SAB ($p < 0.01$).

There was no significant difference between the number of vascular access procedures in the 6 months before (average of 1.19 procedures) or after (average of 1.71 procedures) developing SAB ($p= 0.17$).

MSSA and MRSA bacteraemias were compared in Table 3-1. 15.7% of SAB were caused by MRSA. There were no MRSA bacteraemias in renal transplant recipients ($p=0.04$ compared to MSSA bacteraemias). There was no significant increase in MRSA bacteraemia in patients with TCVC or NTCVC dialysis access. Patients with MRSA bacteraemia had significantly fewer hospital admissions ($p=0.002$) and significantly fewer inpatient days ($p=0.011$) during the first 6 months following first bacteraemia compared to those with MSSA bacteraemia (Table 3-1).

Table 3-1 Comparison of MSSA and MRSA bacteraemia. Data are expressed as value (%) or mean (standard deviation) as appropriate. 1 patient had MSSA and MRSA bacteraemia therefore was excluded from the analysis

Patient group	MSSA bacteraemia n=69 patients n=85 bacteraemias	MRSA bacteraemia n=14 patients n=15 bacteraemias	**P value**
Renal Transplant	4 (4.7)	0	0.040
HD with AVF	15 (17.6)	4 (26.7)	0.458
HD with TCVC	51 (60.0)	8 (53.3)	0.632
HD with NTCVC	13 (15.3)	1 (6.7)	0.252
Peritoneal dialysis	1 (1.2)	0	0.314
No IV access	1 (1.2)	2 (13.3)	0.170
Average no. of admissions in 6 months before first SAB	1.5 (1.3)	0.86 (0.77)	0.113
Average no. of admissions in 6 months after first SAB	2.3 (1.6)	0.93 (0.73)	**0.002**
Inpatient days in 6 months before first SAB	10.8 (11.8)	13.8 (26.8)	0.524
Inpatient days in 6 months after first SAB	17.5 (16.8)	8.0 (9.7)	**0.011**
Average no. of procedures in 6 months before first SAB	1.3 (1.3)	0.9 (1.6)	0.163
Average no. of procedures in 6 months after first SAB	1.7 (1.9)	1.4 (1.6)	0.796
Metastatic infection	11 (15.9)	1 (7.1)	0.396
Death	17 (24.6)	6 (40.0)	0.135

Table 3-2 Comparison of flucloxacillin monotherapy and vancomycin monotherapy in MSSA bacteraemia. Data are expressed as value (%) or mean (standard deviation) as appropriate

Patient group	**Flucloxacillin monotherapy: n = 13 patients**	**Vancomycin monotherapy: n = 18 patients**	**P value**
Renal Transplant	2 (15.4)	2 (11.1)	0.73
HD with AVF	3 (23.0)	3 (16.7)	0.66
HD with TCVC	4 (30.8)	11 (61.1)	0.08
HD with NTCVC	4 (30.8)	2 (11.1)	0.18
Average no. of admissions in 6 months before first SAB	1.77 (0.83)	1.53 (1.29)	0.50
Average no. of admissions in 6 months after first SAB	2.54 (1.98)	2.07 (1.43)	0.58
Inpatient days in 6 months before first SAB	11.15 (8.35)	8.36 (9.74)	0.22
Inpatient days in 6 months after first SAB	16.31 (12.94)	13.71 (8.75	0.68
Average no. of procedures in 6 months before first SAB	1.58 (1.38)	2.07 (1.44)	0.44
Average no. of procedures in 6 months after first SAB	1.08 (1.44)	2.21 (2.05)	0.12
Metastatic infection	2 (15.4)	3 (16.7)	0.92
Death	3 (23.1)	3 (16.7)	0.66

Table 3-3 Comparison of flucloxacillin based regimen and vancomycin based regimen in MSSA bacteraemia. Data are expressed as value (%) or mean (standard deviation) as appropriate

Variable	**Flucloxacillin based regimen:** **n = 29 patients, n = 32 bacteraemias**	**Vancomycin based regimen:** **n =23 patients, n = 28 bacteraemias**	**P value**
Transplant	2 (6.3)	2 (7.1)	0.89
AVF	6 (18.8)	5 (17.9)	0.92
TCVC	16 (50.0)	17 (60.7)	0.40
NTCVC	6 (18.8)	4 (14.3)	0.64
Average no. of admissions before first MSSA bacteraemia	1.48 (1.27)	1.68 (1.56)	0.72
Average no. of admissions after first MSSA bacteraemia	2.62 (1.97)	2.50 (1.47)	0.90
Average no. of inpatient days before first MSSA bacteraemia	10.72 (9.74)	9.36 (10.93)	0.45
Average no. of inpatient days after first MSSA bacteraemia	16.79 (13.82)	13.50 (9.21)	0.57
Average no. of procedures before first MSSA bacteraemia	1.39 (1.32)	1.18 (1.33)	0.53
Average no. of procedures after first MSSA bacteraemia	1.32 (1.59)	2.05 (2.08)	0.17
Metastatic infection	5 (15.6)	4 (14.3)	0.84
Death	6 (20.7)	4 (17.4)	0.63

Table 3-4 Comparison of MSSA bacteraemia by antibiotic duration. Data are expressed as value (%) or mean (standard deviation) as appropriate

Variable	**≤14 days Rx n= 51 patients n=60 bacteraemias**	**>14 days Rx n=16 patients n= 16 bacteraemias**	**P value**
Average no. of admissions before first MSSA bacteraemia	1.37 (1.28)	1.50 (1.03)	0.46
Average no. of admissions after first MSSA bacteraemia	2.06 (1.59)	3.06 (1.91)	0.04
Average no. of inpatient days before first MSSA bacteraemia	11.01 (17.01)	11.88 (19.79)	0.33
Average no. of inpatient days after first MSSA bacteraemia	14.00 (10.97)	20.63 (13.61)	0.06
Average no. of procedures before first MSSA bacteraemia	1.28 (1.46)	1.56 (0.89)	0.12
Average no. of procedures after first MSSA bacteraemia	1.86 (2.07)	12.88 (2.60)	0.16
Metastatic infection	3/51 (5.9%)	5/16 (31.3%)	0.04
Death	13/51 (25.5%)	4/16 (25%)	0.97

Table 3-5 Comparison of *S. aureus* bacteraemia and coagulase negative *Staphylococcus* spp. bacteraemia. Data are expressed as value (%) or mean (standard deviation) as appropriate

Variable	***S. aureus* bacteraemia** **n= 84 patients** **n= 102 bacteraemias**	**Coagulase negative *Staphylococcus* spp bacteraemia** **n= 191 patients** **n= 260 bacteraemias**	**P value**
Average no. of admissions before first bacteraemia	1.42 (2.29)	1.36 (1.38)	0.50
Average no. of admissions after first bacteraemia	2.08 (1.64)	1.58 (1.51)	0.008
Average no. of inpatient days before first bacteraemia	11.26 (15.13)	9.74 (13.54)	0.29
Average no. of inpatient days after first bacteraemia	15.79 (16.10)	13.87 (16.73)	0.07
Average no. of procedures before first bacteraemia	1.19 (1.32)	1.06 (1.23)	0.49
Average no. of procedures after first bacteraemia	1.71 (1.93)	1.05 (1.03)	0.007
Metastatic infection	12/84 (14.5%)	7/191 (3.7%)	0.009
Death	23/84 (27.4%)	52/191 (27.2%)	0.98

There were no significant differences in demographics or outcomes between patients treated with flucloxacillin monotherapy or vancomycin monotherapy (Table 3-2) or between those treated with a flucloxacillin-based regimen compared to a vancomycin-based regimen (Table 3-3). This data contrasts with the previous clinical viewpoint that a vancomycin based regimen reduced inpatient days, although this hypothesis has not been

proven by observational research (208). Patients treated with a prolonged antibiotic course for MSSA bacteraemia had more hospital admissions with an increased incidence of metastatic infection (p=0.04) (Table 3-4). There was significantly more metastatic infection in those with *S. aureus* bacteraemia compared to coagulase negative *Staphylococcus* spp (p=0.009). There was no significant difference in mortality rates (Table 3-5). This was surprising, and may be related to the high co-morbidity of the study population.

17 patients had more than a single episode of *S. aureus* bacteraemia. All of these patients required regular HD compared to 54/67 (80.6%) of the remainder of this population (p= 0.000). Two (11.8%) of the patients with more than a single episode of bacteraemia had metastatic infection compared with nine (16.7%) of the remainder of the *S. aureus* bacteraemia cohort.

3.3.3 Staphylococcus spp. bacteraemia in regular haemodialysis patients only

Bacteraemia in regular HD patients can be expressed per 1000 dialysis days or per 1000 catheter days in those with tunneled central venous catheters (TCVC). From Scottish Renal Registry data TCVC use in prevalent HD patients in the Glasgow Renal and Transplant Unit was 27% and 26%, in 2010 and 2011 respectively, confirmed by unit specific data collection from the vascular access team (personal communication, Emma Aitken). No data were available for NTCVC days.

198 patients from this cohort were receiving long term HD at the time of bacteraemia. This accounts for 314 bacteraemias with 88 *S. aureus* bacteraemias (including 15 MRSA bacteraemias) and 215 coagulase negative staphylococcal bacteraemias. Total bacteraemia rate was 0.87 per 1000 dialysis days for all bacteraemias with 0.21 per 1000 dialysis days for MSSA bacteraemias, 0.04 MRSA bacteraemias per 1000 dialysis days and 0.62 coagulase negative staphylococcal bacteraemias per 1000 dialysis days.

TCVC associated bacteraemia prevalence was 2.02/1000 catheter days. MSSA bacteraemia occurred in 0.46/1000 catheter days, MRSA bacteraemia in 0.08/1000 catheter days and coagulase negative staphylococcal bacteraemia in 1.48/1000 catheter days.

AVF associated bacteraemia was 0.21/1000 AVF days. MSSA bacteraemia occurred in 0.06/1000 AVF days, MRSA bacteraemia 0.02/1000 AVF days and coagulase negative staphylococcal bacteraemia 0.13/1000 AVF days.

Standard treatment of staphylococcal bacteraemia during the study period was difficult to define and depended on the perceived source of infection, bacteria cultured, dialysis access and individual clinician preference.

Table 3-6 illustrates bacteraemia rates in the prevalent HD patient population directly comparing TCVC and AVF bacteraemia. Bacteraemia secondary to MSSA and coagulase negative staphylococci were significantly increased in those patients dialysing via TCVC.

Table 3-6 Comparison of form of haemodialysis access by *Staphylococcus* spp. bacteraemia

	All patients bacteraemia /1000 HD days)	**TCVC patients (bacteraemia /1000 TCVC days)**	**AVF patients (bacteraemia /1000 AVF days)**	**P value**
MSSA	0.21	0.46	0.06	0.000
MRSA	0.04	0.08	0.02	0.024
Coagulase negative *Staphylococcus*	0.62	1.48	0.13	0.000
All *Staphylococcus* spp.	0.87	2.02	0.21	0.000

Regular HD via a TCVC was significantly associated with bacteraemia due to all staphylococcal species. Coagulase negative staphylococci rarely cause a sepsis syndrome in an AVF population but can be pathogenic in those with indwelling lines, predominantly due to the effect of biofilm as discussed in Chapter 1.

3.3.4 Spa gene typing of S. aureus bacteraemias

Spa gene typing was performed as routine for all *S. aureus* bacteraemia specimens in the Scottish MRSA reference laboratory. This allows relationships between different *S. aureus* strains according to the polymorphic region of the protein A gene to be represented visually using a Based Upon Repeat Pattern (BURP) diagram. The spacing between linked *spa* types and between unlinked *spa* types provides no information concerning the genetic distance between them. The *spa* type with the highest founder-score is defined founder of the cluster (blue colour). Subfounders are the *spa* types with the second highest founder-score and are labelled in yellow. If two or more *spa* types exhibit the same highest founder-score, they are all coloured in blue.

Spa typing was available for 96% of *S. aureus* isolates. There were 7 clusters with 11 singletons. Clusters 1-3 accounted for 28% of all isolates and all clusters are illustrated in Figure 3-1. Discriminatory Power was 0.97 (95% confidence interval 0.956-0.983). There was no difference in Discriminatory Power when samples originating from regular HD patients only were analysed. The Discriminatory Power is the average probability that the typing system will assign a different type to two unrelated strains randomly sampled in the microbial population.

17 patients had more than a single staphylococcal bacteraemia. These were all regular HD patients. In 14 of these patients (77.8%) the subsequent *spa* type was identical to the original bacteraemia.

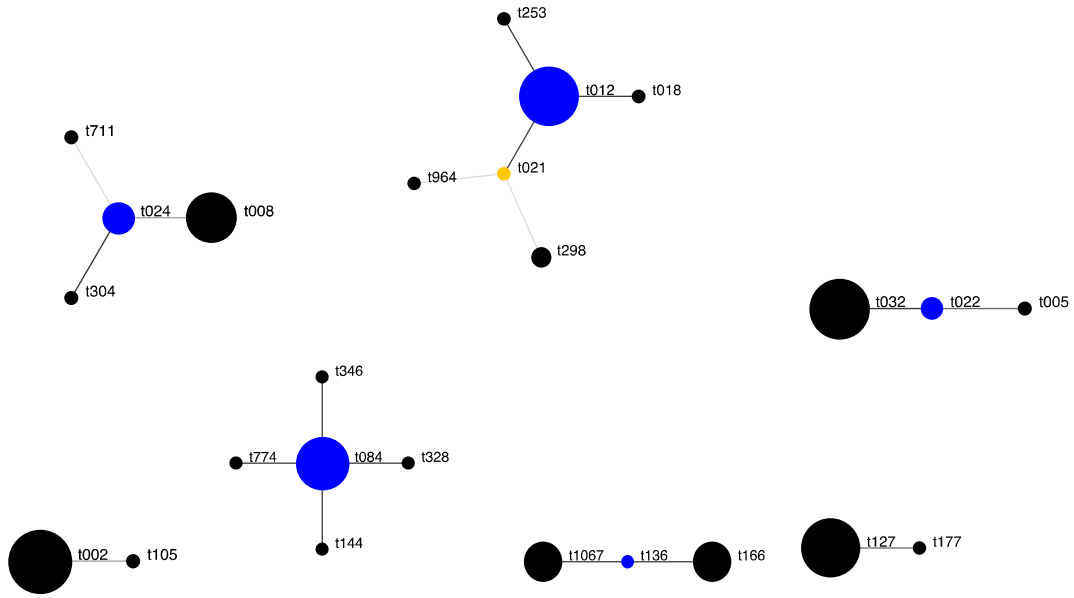


Figure 3-1 Population snapshot of the 53 *S. aureus* strains after BURP grouping. The *spa* type with the highest founder-score is defined founder of the cluster (blue colour). Subfounders are the *spa* types with the second highest founder-score and are labelled in yellow

3.4 Discussion

Staphylococcal bacteraemia remains common in the renal unit, particularly among our HD patients and the highest bacteraemia rates (2.02/1000 days) were observed in HD patients dialysing via a TCVC. This pattern of prevalence of bacteraemia in HD patients is similar to the rates documented in the renal patient population in the same unit in 2004-2005 (0.3/1000 dialysis days in HD patients with arteriovenous fistulae, 1.8/1000 dialysis days in patients with TCVC and 6.3/1000 dialysis days in patients with a NTCVC) (99). Given the previously documented high incidence of Staphylococcal bacteraemia in the Glasgow HD population a range of measures designed to reduce infection were in place at the time of this audit. TCVC were inserted by a core team of specialist nurses and interventional radiologists in a controlled environment under strict aseptic conditions. Non-tunnelled central venous catheter (NTCVC) insertion was mostly performed by renal trainees under aseptic conditions in the ward environment or a treatment room. A chlorhexidine-impregnated "Biopatch™" was placed at the catheter exit site. A single retrospective study reported a reduction in exit site infection but no reduction in catheter related bacteraemia was associated with this intervention (211). It is disappointing that the bacteraemia rates

were not improved in this study in 2010-2011 despite the routine use of a range of measures to prevent bacteraemia in HD patients.

In the historic literature a definition of bacteraemia required documentation of a systemic inflammatory response as well as positive blood culture results. This was not a criterion in this series as bacteraemia may not be associated with fever or neutrophilia in renal patients. Inclusion of all episodes of positive blood cultures as episodes of bacteraemia in this study may overestimate the true prevalence of bacteraemia, especially the incidence of coagulase negative staphylococcal bacteraemia due to contamination when blood sampling or colonisation of the HD catheter due to biofilm formation.

The incidence of coagulase negative *Staphylococcus* spp. bacteraemia may be difficult to correlate with clinical significance in this study population as not all of these episodes were associated with clinical evidence of sepsis. Nevertheless coagulase negative *Staphylococcus* spp bacteraemia, defined as the presence of a positive blood culture only, was associated with admission rates, number of inpatient days and mortality rates in this study which were not significantly different from *S. aureus* bacteraemia (Table 3-5). The high mortality rate after Staphylococcal bacteraemia in the HD population may be related to this patient population's high cardiovascular and other co-morbidity (99). As expected *S. aureus* bacteraemia was associated with a significantly higher risk of metastatic infection than coagulase negative staphylococcal spp bacteraemia (Table 3-5). *S. aureus* bacteraemia is known to confer a major economic burden on renal units, with an estimated cost of up to $32000 for each patient who is hospitalised for bacteraemia in the US (212, 213).

Outcomes with MRSA and MSSA bacteraemia in this cohort contrast with other published literature (214) (215). MRSA bacteraemia accounted for 15.7% of all SAB. This is lower than reported in historical series of HD patients or hospitalized patients. 109 HD patients with SAB from 3 German hospitals between 1999 and 2005 were reviewed in an attempt to assess variation in demographics and outcomes between community-acquired and nosocomial infection. 25.7% patients in this cohort had MRSA bacteraemia. Mortality rates in MRSA and MSSA bacteraemias were 32.1% and 30.9%, respectively and the average treatment costs for patients with MSSA bacteraemia were <50% of patients with MRSA bacteraemia (€10,573 vs. €24,931, $p < 0.05$). An American study, in which 37.8% of the HD patients had MRSA bacteraemia, reported that there was an increased risk of death associated with MRSA bacteraemia. There were higher adjusted costs for the initial

hospitalization for MRSA bacteraemia ($21,251 vs $13,978; p = 0.012) and after 12 weeks ($25,518 vs $17,354; p = 0.015) (215). MRSA bacteraemia in general has been falling over the past 10 years and therefore the current series may be more comparable with present day bacteraemia rates.

There is little information on how best to treat *S. aureus* bacteraemia in renal patients, especially HD patients. The largest available dataset from the USA analysed data from 293 094 outpatient HD patients over the 4 year period, January 1st 2006 until December 31st 2010 (208). In this population, the rate of MSSA bacteraemia was 2.1 per 100 outpatient-years, and rate of MRSA bacteraemia was 1.9 per 100 outpatient-years. This retrospective data showed that the 1st generation cephalosporin, cefalozin was associated with significantly reduced risk of hospitalization or death compared to vancomycin (hazard ratio=0.50, 95% CI=0.35–0.73). This study was limited because it was retrospective and so information regarding inpatient care and all additional interventions that were required to treat infection such as catheter removal or changes was not available. In addition, vancomycin levels were not consistently recorded raising the possibility that subtherapeutic vancomycin titres may have contributed to the poorer outcomes with vancomycin. The increased use of high flux haemodialysis and haemodiafiltration results in increased vancomycin removal requiring adjustment of dosage regimens (216).

The MRSA bacteraemia rate for HD patients from this unit is comparable to other large renal units in the UK as illustrated by UK Renal Registry Report data (Figure 3-2, below). Data are expressed per 100 prevalent HD patients. The Glasgow Renal and Transplant Unit has had approximately 650 regular HD patients throughout 2010 and 2011 and so the MRSA bacteraemia rate per 100 prevalent HD patients in 2010 and 2011 was 1.15.

MSSA bacteraemia was also recorded for the first time in the 14th Annual UK Renal Registry Report in 2012 and the total MSSA bacteraemia was reported as 1.7 per 100 prevalent HD patients in X of the total renal units in England (217). There were 5.61 MSSA bacteraemias per 100 prevalent HD patients in this Glasgow population but this needs to be interpreted in the context of a probable incomplete dataset from the UK Renal Registry given the lack of mandatory reporting in this population at the time of data collection.

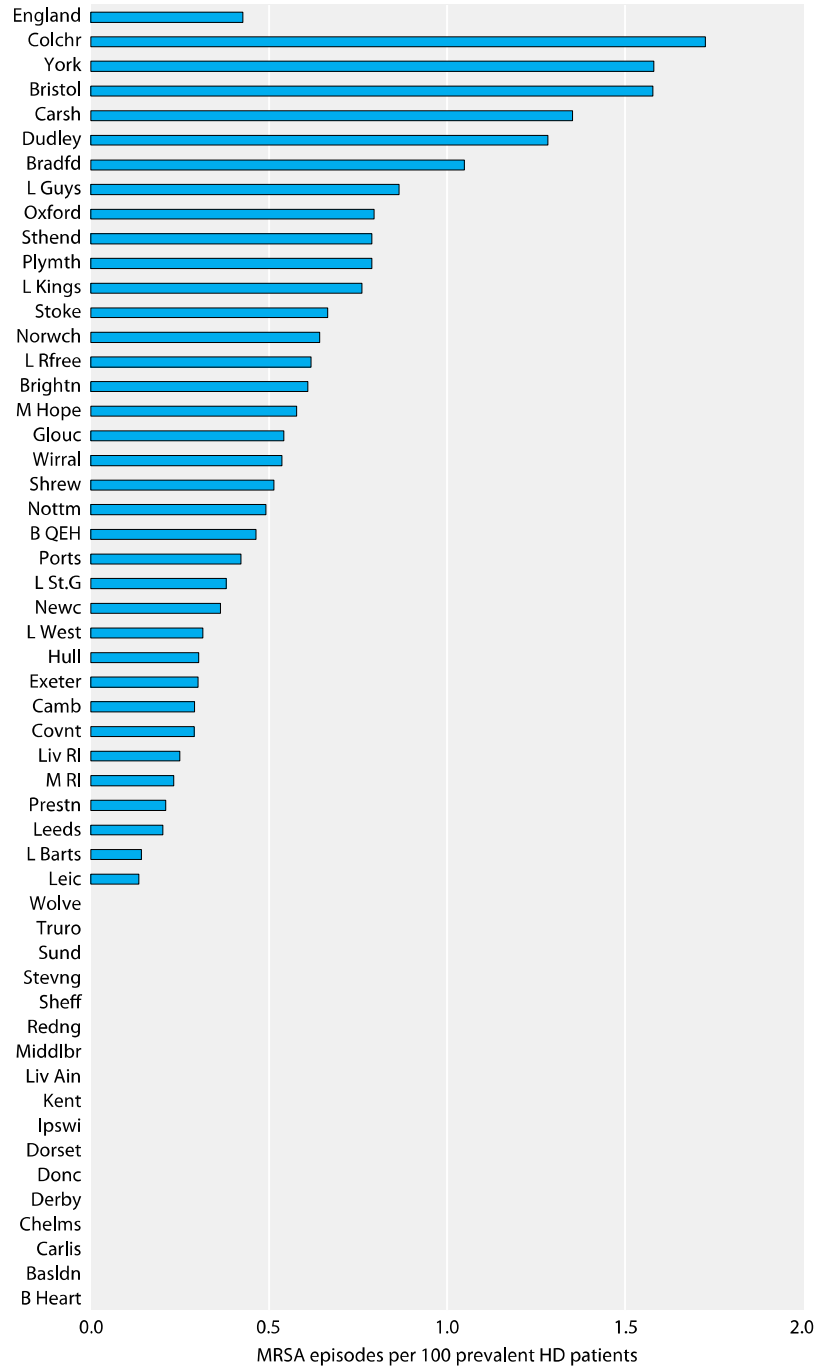


Figure 3-2 Figure 3-4 MRSA bacteraemia rate per 100 prevalent HD patients by renal centre: 1/4/2009 to 31/3/2010 For each centre the rate per 100 prevalent HD patients as reported 31/12/2009 is provided (217).
The overall rate for England is provided at the top of the graph

Data obtained from spa gene analysis is difficult to interpret on an epidemiological level due to the relatively small number of samples. Recurrent infection with *S. aureus* containing the same spa gene supports existing evidence of individuals predominantly carrying a single strain of *S. aureus* despite attempts at eradication.

3.5 Conclusion

Staphylococcus spp. sepsis is common in the renal unit and predominantly affects regular HD patients. It is strongly associated with the use of central venous catheterisation for vascular access and catheter related staphylococcal bacteraemia is associated with a high incidence of metastatic infection. The key objective is therefore to reduce the proportion of HD patients using central venous catheters for vascular access. An AVF is the preferred mode of vascular access for HD and effective bundles of care are already in place to reduce the risk of bacteraemia in patients using an AVF. Despite long-term efforts at maximizing the percentage of HD patients using an AVF for vascular access, many patients unfortunately continue to require TCVC for vascular access for HD.

UK guidelines advise a target of 85% prevalence of AVF or arteriovenous graft use, however only a minority of renal units are currently achieving this (218). High quality observational data has repeatedly found a clinically and statistically significant increase in mortality in patients undergoing haemodialysis via a TCVC compared to AVF. Registry-based data over three years with a maximum follow up of 36 months found a increased mortality associated with TCVC use. Survival analysis using unadjusted data and data adjusted for comorbidities found significantly increased risk of death with 28.2% patients dialysing only via AVF or arteriovenous grafts during follow up compared to 45.2% of those dialysing via TCVC (unadjusted) (219).

3.6 Suggestions for further research

Further investigation of the optimal bundle of care for prevention of catheter related infection is required to reduce hospitalisation and mortality rates in this comorbid population. The optimum duration of antimicrobial therapy and antibiotic choice to adequately treat sepsis, limit metastatic infection and minimise antibiotic related side effects in renal patients is yet to be determined and should be studied further.

Future research will focus on prevention of infection with alternative methods of vascular access such as increased use of arteriovenous grafts and modifications to TCVCs to reduce biofilm formation. Antimicrobial line locks have been studied with evidence of a reduction in bacteraemia rates, however there are concerns that antibiotics used in the form

of line lock would promote antimicrobial resistance as has been seen in clinical practice in the USA(220).

Increased patient involvement in their own management may also improve care of their vascular access device or AVF resulting in decreased infectious complications. Attention to self care regimens, patient education and patient empowerment, as part of a wider culture, may help to improve outcomes. Such interventions are underrepresented in the current literature.

Chapter 4: Observational study of the prevalence of *Staphylococcus aureus* toxin gene positivity in samples from different patient populations including a renal dialysis unit in Glasgow, UK

4.1 Introduction

Staphylococcus aureus isolates carrying the Panton-Valentine leucocidin (PVL) toxin gene have been responsible for outbreaks of community acquired severe invasive disease in the recent past (221) (222) (223). It causes cell injury, and therefore disease, by activating neutrophils before creating lytic pores damaging the cell membrane (224). Injection of purified PVL toxin induces release of histamine, enzymes such as lysozyme, chemotactic factors, and oxygen metabolites from neutrophils. Injection of purified PVL toxin intradermally in rabbits resulted in severe inflammatory lesions with capillary dilatation, chemotaxis, neutrophil infiltration and skin necrosis (225). Disease resulting from PVL positive *S. aureus* characteristically causes severe skin infections and a necrotising pneumonia (226). Case series of those with pneumonia secondary to PVL positive S. *aureus* found that it was commonly preceded by a viral type illness. One possible mechanism for this is that an initial viral lung infection could result in desquamation of ciliated and secretory cells, allowing bacterial adhesion to basal epithelial cells (227). It is known that the prevalence of PVL positive *S. aureus* colonisation varies in different geographical areas (228) (229).

Toxic shock toxin (TSST) has also been implicated in community acquired MRSA and is a superantigen. Superantigens are able to bind directly to the major histocompatibility complex on the surface of antigen-presenting cells outside the antigen-binding groove. They cross-link with T cell receptors resulting in increased T cells activation (230). Toxic shock toxin is produced by 5-25% of *S. aureus* isolates. It causes toxic shock syndrome by stimulating the release of large amounts of interleukin-1, interleukin-2 and tumour necrosis factor (231). Toxic shock syndrome (TSS) was first described in 1978 and was in association with highly absorbent tampons (232). Once these were identified as being a risk factor for TSS they were removed from the market and the incidence has declined since. It manifests as the development of septic shock with a desquamating skin rash.

Exfoliative toxins A and B (ETA and ETB) mostly cause disease in children and result in a spectrum of disease ranging from localised blisters to generalised exfoliation affecting the entire body surface area (230). Around 5% of *S. aureus* strains produce exfoliative toxin. They are responsible for the staphylococcal scalded skin syndrome (SSSS). Although

SSSS predominantly affects children, adults with renal impairment are also at increased risk. SSSS in adults carries a greater than 50% mortality (233).

Toxin gene positivity including PVL positivity has been associated with increased virulence and disease severity associated with *S. aureus,* however this is now disputed (234). It has not been a consistent finding in observational human studies, animal models and in vitro research and deletion of the genes encoding the PVL toxin gene has not been shown to reduce virulence in animal models(224, 225, 235-240). Recent research suggests that PVL may play a role in activation of the innate immune response, and therefore function in host recognition of *S. aureus* infection and facilitate resolution of disease (241).

Prior to toxin gene analysis and epidemiological typing, *S. aureus* Methicillin resistance must be determined. Methicillin resistance in *S. aureus* is conferred by the *mecA* gene, which encodes a membrane protein that has a low penicillin-binding affinity (penicillin-binding protein 2a). *mecA* is located within a mobile genetic element named staphylococcal chromosomal cassette *mec* (SCC*mec*).

Isolates of *S. aureus* can be characterised by *spa* typing. This involves DNA sequencing of short sequence repeats in the polymorphic X region of the protein A gene (*spa*) of *S. aureus*. Each new base composition of a repeat is assigned an alpha-numerical code and the repeat succession determines the *spa* type. BURP (Based upon repeat pattern) diagrams– as implemented in the StaphType software v. 1.5 (Ridom GmbH, Würzburg, Germany) can be used to cluster *spa* types and therefore infer their clonal relatedness. (124) *Spa* typing is used world-wide for reliable, accurate and discriminatory typing of *S. aureus* (both MSSA and MRSA). In commonly occurring lineages, DNA sequencing of the *spa* gene allows presumptive identification of healthcare- and community-associated MRSA strains identified in the UK and multi locus sequence typing (MLST) clonal complex designation. Certain toxin genes have been associated with particular *spa* types.

The USA300 clone is associated with community acquired MRSA. It has increased virulence and transmission and is characteristically *spa* t008 or t024. Isolates with the *spa* type t008 are associated with PVL positivity. The USA300 strain was first reported in 2000 as causing skin and soft tissue infections amongst American sportsmen and prisoners. It is the most prevalent strain of community acquired MRSA and may account for up to 50% of those colonised with MRSA in the community setting in the US (242) (243).

The prevalence of PVL, toxic shock toxin and exfoliative toxins in *S. aureus* in the community and haemodialysis patients is unknown. In Scotland, these toxin genes are screened for in all *S. aureus* positive blood culture isolates which are routinely submitted to the Scottish MRSA Reference Laboratory (SMRSARL). However, not all isolates isolated from other sites are submitted, and testing for toxins is performed on request.

This observational cohort study assesses the prevalence of PVL, TSST, ETA and ETB toxin genes in *S. aureus* from 4 different populations.

- Assessment of nasal carriage of methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA) from:
    - All regular haemodialysis patients at Glasgow Royal Infirmary and Stobhill Hospital, Glasgow
    - "healthy volunteers" from an orthopaedic preoperative assessment clinic at Glasgow Royal Infirmary
- Isolates originating from GP practices of skin infections
- *S. aureus* positive blood cultures

Laboratory analysis was at the MRSA reference laboratory for Scotland. Isolates from the GP practices gave an estimate of community based disease. *Spa* type was also ascertained in order to allow epidemiological analysis of *S. aureus* specimens.

4.2 Methods

4.2.1 Laboratory assays

S. aureus ID (SAID; bioMérieux, La Balme Les Grottes, France) chromogenic agar plates were used for rapid and reliable identification of *S. aureus* species. On SAID agar, *S. aureus* forms distinctive green colonies due to production of α-glucosidase (Figure 4-1). Other staphylococci generally form white colonies but occasionally produce pink colonies due to the hydrolysis of a second chromogenic substrate for β-glucosidase (244).

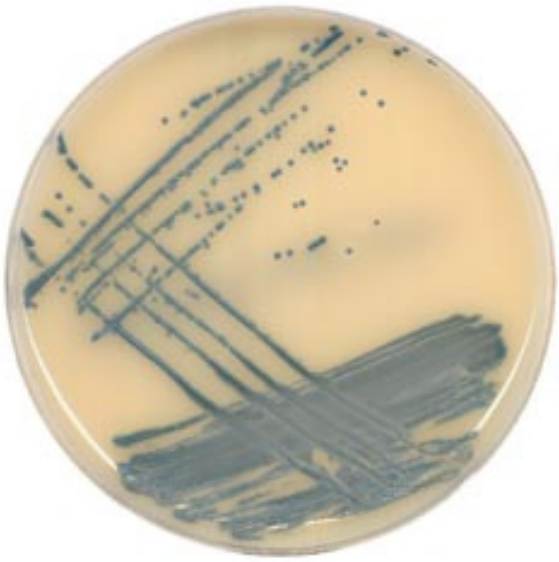


Figure 4-1 *S. aureus* culture on SAID chromogenic agar plate

Latex agglutination testing (Figure 4-2) (Staphaureux Plus, Thermoscientific) confirmed the presence of a *S. aureus* colony. Yellow latex particles coated with human fibrinogen for detection of clumping factor and coated with specific IgG for detection of protein A and surface antigens characteristic of MRSA and MSSA strains are visible in the bottom left and top middle areas of the card illustrated below.

Figure 4-2 Latex agglutination testing kit

Standard horse blood agar plates were inoculated with a single colony and the susceptibility of the organism against cefotaxime, as a measure of methicillin resistance, and mupirocin antibiotic discs was assessed in line with Clinical and Laboratory Standards Institute Guidelines.

Polymerase chain reaction testing followed by gel electrophoresis was performed for presence via the method described below. These identify nuc, MecA and MupA genes via the method below which detect *S. aureus*, MRSA and mupirocin resistance respectively. The same specimens were tested for the presence of PVL, TSST, ETA and ETB toxin genes using the same method using appropriate primers.

4.2.2 DNA Extraction method

- Label the lids of autoclaved 0.5ml microtubes ((1 – x) depending on number of isolates on the worksheet) and place the labelled tubes in a polystyrene float
- Include positive and negative controls with each run
- Tube 1 should always be a water control
- Add 50µl NET buffer to each microtube using a pipette and filter tip
- Add 10µl Achromopeptidase to each microtube using a pipette and filter tip
- Using a 1µl plastic loop suspend 1-2 colonies of the appropriate culture into each tube
- Place the polystyrene float into the 50˚C waterbath, and incubate for 10-15 minutes

4.2.3 Preparation of PCR Reaction Mix

- Place multiwell plate on frozen block to ensure reagents remain cool
- Add 23µl of PCR Master Mix to each well of the multiwell plate as necessary using a pipette and filter tip
- Remove polystyrene float from water bath
- Add 2µl of lysed cell suspension to the appropriate well of the multiwell plate using a pipette and fresh filter tip for each sample
- Place the multiwell plate onto the MWG plate sealer block
- Place foil seal on top of the multiwell plate with the white side up/silver side down

- Press down on the handle until you feel contact between the upper heated plate and the multiwell plate then press down a little further, and hold contact for at least 15 seconds
- Release the handle slowly
- The plate is now ready to be placed in the thermal cycler

4.2.4 Thermal Cycle

- Place the multiwell plate or individual tubes in the thermal cycler ensuring lids of individual tubes are securely closed
- Press the lid down firmly to close
- Select the desired PCR cycle
- When a run has finished, the timer is at zero. Press the Red **STOP** button until the screen reads **'MY JOB IS DONE'**, open the lid and remove samples

4.2.5 Electrophoresis Gel Preparation

- Depending on the number of isolates being tested and the PCR being carried out, select the appropriate size of gel
    - Up to 8 isolates: Mini Gel
    - Up to 14 isolates: Small Gel
    - Up to 30 isolates: Large Gel – either a **20 well** or **30 well** comb can be used in large gels
- Into a 500ml Duran bottle, place the correct weight of agarose, and volume of distilled water and x10 TBE (to result in a 1.5% agarose gel) according to the table below:

Table 4-1 Composition of electrophoresis gel depending on size required

	Agarose	Distilled Water	X 10 TBE* Buffer
Mini	1.5 g	105 ml	5 ml
Small	1.5 g	105 ml	5 ml
Large	3.0 g	190 ml	10 ml

* Tris/Borate/EDTA

- Screw the cap onto the bottle very loosely
- Place in microwave
- Heat at full power for 2 minutes
- After 1 minute - stop the microwave by opening the door, and swirl the bottle whilst still within the microwave
- Return the bottle to the microwave and continue heating
- Cool by placing in 50˚C waterbath for 15 minutes **or** by running cold water from tap over bottle with constant swirling
- Prepare gel cast by taping both ends with white tape
- Pour the gel, and then place comb(s) in the notches
- Allow agarose to set for 20-30 minutes

4.2.6 Electrophoresis Tank Preparation

- Prepare the appropriate volume of buffer in a 2L measuring cylinder for the tank required as follows and pour carefully from the measuring cylinder into the tank

Table 4-2 Composition of buffer required for electrophoresis tank depending on size

	Tank	Distilled Water	x 10 TBE* buffer
Mini	Mini Tank	190	10
Small	Horizon 11:14	950	50
Large	Horizon 20:25	1900	100

* Tris/Borate/EDTA

- Place the agarose gel into the tank with the wells towards the negative (black) electrode and carefully remove comb(s) from the agarose gel

4.2.7 Gel Electrophoresis

- Starting at the well closest to the front of the tank, load 6µl PCR product to each well as appropriate
- Plug in black lead to black electrode on tank and black lead into black electrode on power pack.
- Plug in red lead to red electrode on tank and red lead into red electrode on power pack.
- Set voltage and running time according to gel size
- Once complete, switch off the power pack and remove both red and black leads from the tank and power pack
-

4.2.8 Ethidium Bromide Staining

This is carried out in designated area due to its carcinogenic and teratogenic properties

- Remove gel and gel cast from tank carefully
- Slide the gel (not the cast) into an ethidium bromide dedicated staining tray
- Pour 0.0001% ethidium bromide from brown shatterproof bottle over the gel with the gel being completely immersed in stain.
- Leave to stain for 15-20 minutes
- Carefully remove gel from ethidium bromide using plastic gel scoop
- Place gel in a fresh tray containing tap water to remove residual ethidium bromide
- Return the ethidium bromide to the brown shatterproof bottle by pouring from the tray through the dedicated funnel
- Photograph the gel and store image (Figure 4-3)
- Discard the gel into a “Solid” toxic waste container

4.2.9 Quality control measures

- The water control should have no presence of DNA

- The positive control band(s) must be present
- The negative control band(s) must be absent
- Internal controls should be positive for all isolates. A negative result may indicate extraction failure
- If there is failure of any control, then the assay must be repeated
- All batch/lot numbers of reagents should be recorded on the worksheet
- Work-flow should be unidirectional i.e. from clean areas to contaminated areas, but not from contaminated areas to clean areas.

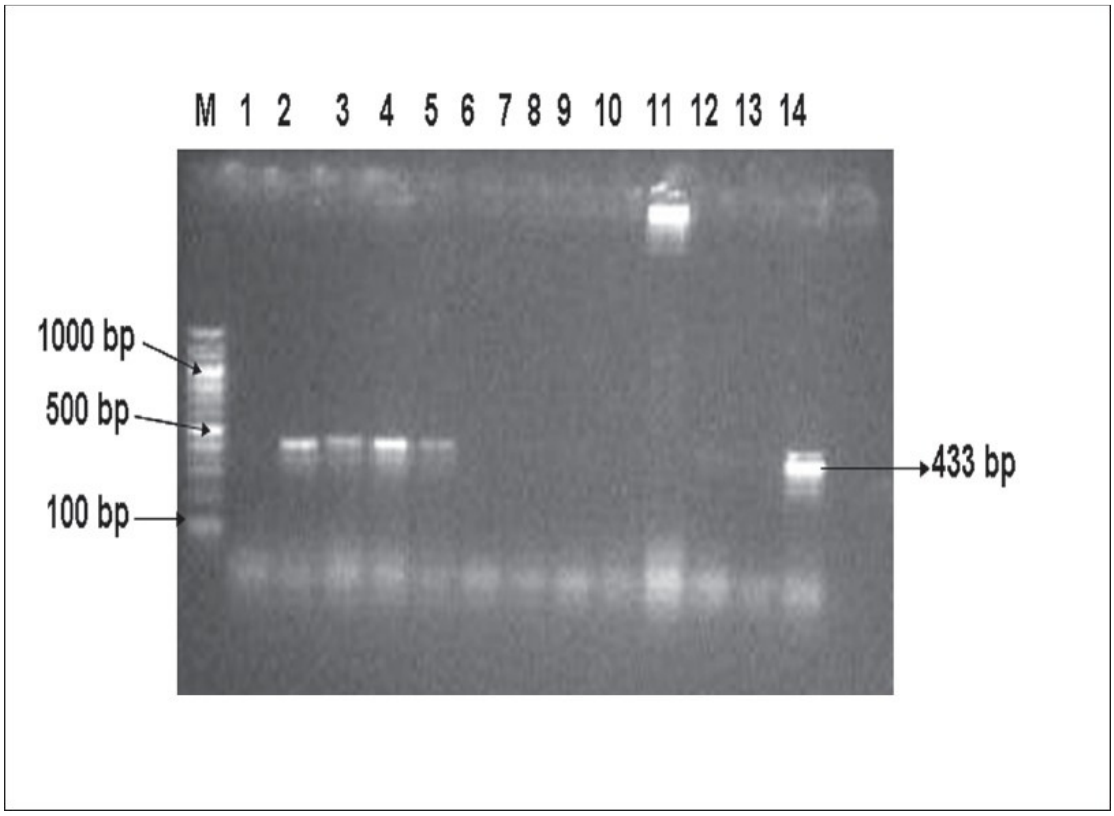


Figure 4-3 Example of protein gel electrophoresis confirming PVL positivity of samples 2-5 with control samples at position 1 and 14.

Spa testing was performed by GATAC Biotech (Germany) after PCR amplification in house using Ridom primers.

The software Ridom StaphType^{TM} (Ridom GmbH, Würzburg, Germany) was used for *spa* sequence analysis including the generation of BURP diagrams.

4.2.10 Study population

Group 1: Haemodialysis patients at Glasgow Royal Infirmary and Stobhill Hospital, Glasgow, aged over 18 and able to give informed consent. These patients were prospectively screened using a nasal swab into charcoal medium. Basic demographic data was collected.

Group 2: Patients attending the preoperative assessment clinic for elective orthopedic surgery at Glasgow Royal Infirmary who were aged over 18 and able to give informed consent. These patients were prospectively screened using a nasal swab into charcoal medium. Basic demographic data was collected

Group 3: Community skin swabs from GP practices in Greater Glasgow and Clyde were cultured in the conventional fashion in the general microbiology laboratory in Glasgow Royal Infirmary. If S. *aureus* was isolated, further analysis as described above was instituted. The origin of the swab and character of the illness was collected.

Group 4: Blood cultures were processed in the conventional fashion by the general microbiology laboratory in Glasgow Royal Infirmary. If *S. aureus* was isolated they underwent further analysis as described above as routine by the MRSA reference laboratory

4.2.11 Statistical analyses

Minitab 13.1 ^{TM} was used for data analysis. The data were not normally distributed and therefore the Mann Whitney Test for non parametric data and 2 Sample T Test were used where appropriate.

4.2.12 Indemnity and ethical approval

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and was healthcare related research covered by NHS indemnity. It was approved by the local Research and Development department and by NHS Greater Glasgow and Clyde research and ethics committee, (reference 10/S0701/65), on the 2nd November 2010.

4.3 Results

148 Haemodialysis patients and 125 healthy patients were tested for *S. aureus* nasal colonisation. 58 skin swabs positive for *S. aureus* were identified from the general microbiology laboratory in a 3 month period giving an estimate of community based disease and 64 blood cultures were identified as being positive for *S. aureus* over a 6 month period from the same geographical area.

4.3.1 Comparison of haemodialysis patients and healthy controls

There was no significant difference in age between the haemodialysis patients and healthy controls however there were significantly more males in the haemodialysis population with 57.9% males in the haemodialysis population compared to 43.2% males in the "healthy" population. There was no significant difference in age between those colonised with *S. aureus* and those without *S. aureus* colonisation in either the haemodialysis or healthy control groups. Only 4 of 44 haemodialysis patients with *S. aureus* colonisation were colonised with MRSA and only 1 of the healthy controls were colonised with MRSA as illustrated by Table 4-3.

Table 4-3 Characteristics of prospectively screened patients (Groups 1 and 2)

	Haemodialysis patients (Group 1)	**Healthy patients (Group 2)**	**P value**
Number of patients screened	145	125	n/a
Mean age (IQR)*	61.17 (49.21, 74.61)	58.33 (47.31, 72.07)	0.15
No. (%) males	84 (57.9)	54 (43.2)	0.015
No. *S. aureus* (% of total screened)	44 (30.3)	34 (27.2)	0.56
No. MRSA (% of *S. aureus* positive)	4 (9.1)	1 (2.9)	0.24
Mean age (IQR) colonised *with S. aureus* (in years)	59.06 (52.68, 73.76)	61.10 (53.89, 72.24)	0.83
Mean age (IQR) not colonised with *S. aureus* (in years)	62.10 (48.16, 75.34)	57.11 (46.26, 71.98)	0.06

*Interquartile range

4.3.2 Comparison of community infections and bacteraemias

58 patients were identified as having a skin infection caused by *S. aureus* during a 2 month period in 2011 with 64 patients having *S. aureus* bacteraemia during a 6 month period from December 2010 from the same geographical area. There was no significant difference in age or MRSA infection however there were significantly more males in the bacteraemia group as illustrated by Table 4-4.

Table 4-4 Characteristics of infected patients (Groups 3 and 4)

	Community *S. aureus* infections (Group 3)	***S. aureus* bacteraemia (Group 4)**	**P value**
Number of patients	58	64	n/a
Mean age (IQR)	58.59 (41.59, 74.29)	64.33 (53.38, 73.05)	0.11
No (%) male	22 (37.9)	43 (66.1)	0.001
No. MRSA (% of *S. aureus* positive)	8 (13.8)	14 (21.9)	0.24

4.3.3 Staphylococcus aureus colonisation compared to infection

There was no statistically significant difference between the ages of those colonised with *S. aureus* (Groups 1 and 2) compared to those with *S. aureus* infection (Groups 3 and 4) with mean ages being 59.95 years and 61.60 years respectively (Table 4-5). Distribution of ages of those with *S. aureus* infection is illustrated by Figure 4-4.

Table 4-5 Comparison of *S. aureus* colonised patients compared to S. aureus infected patients

	***S. aureus* colonisation (Groups 1 and 2)**	***S. aureus* infection (Groups 3 and 4)**	**P value**
Number of patients	78	122	n/a
Mean age (IQR)	59.95 (53.42, 73.49)	61.60 (47.79, 75.99)	0.57
No (%) male	45 (57.7)	65 (53.3)	0.54
No. MRSA (% *S. aureus* positive	5 (6.4)	22 (18.0)	0.009

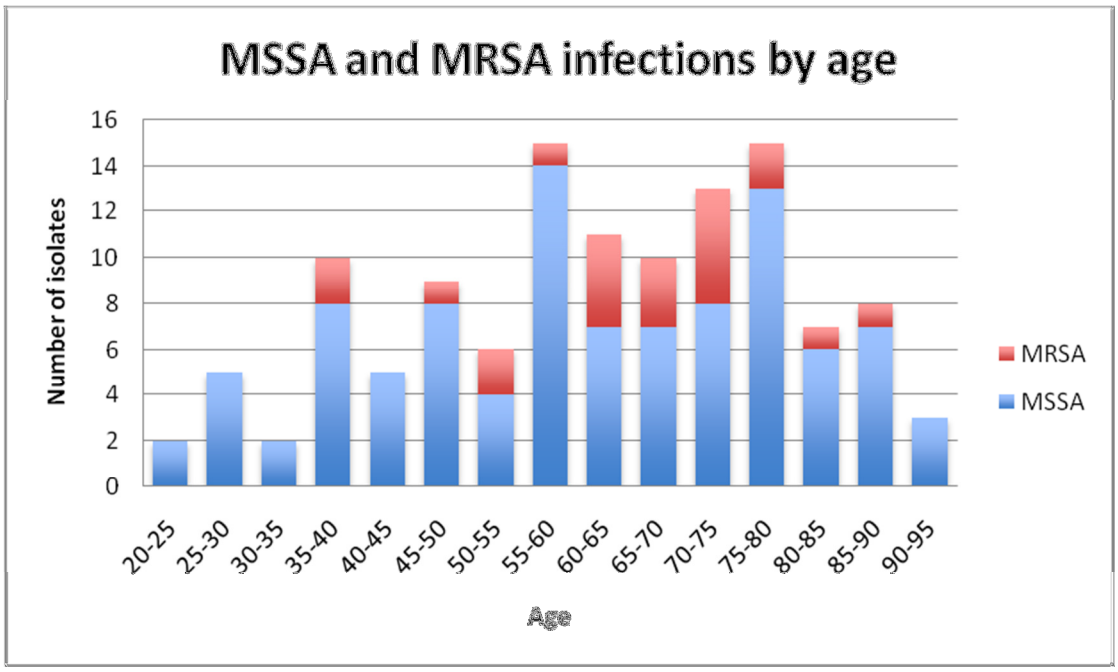


Figure 4-4 Ages of those with *S. aureus* infection of skin or blood (Groups 3 and 4)

4.3.4 Toxin gene positivity

There were toxin genes present in 15.0% of all isolates positive for *S. aureus* and in 7.6% of all isolates in total. There was a single isolate containing the PVL toxin gene from a *S. aureus* positive blood culture. This *S. aureus* was methicillin sensitive. The healthy patients (Group 2) tended to have fewer toxin genes compared to the remainder of the isolates, however this did not reach statistical significance given the small numbers involved. Table 4-6 summarises toxin gene positivity in all populations.

Table 4-6 Summary of toxin gene positivity in all specimens

	No. *S. aureus* (% total screened)	**No. MRSA (% *S. aureus* positive)**	**TSST positive (% positive)**	**ETA or B positive (% positive)**	**PVL positive (% positive)**
Haemodialysis patients (Group 1)	43 (29.7)	4 (9.8)	4 (9.8)	3 (7.0)	0
Healthy patients (Group 2)	30 (24.0)	1 (2.8)	0	3 (10)	0
Community swabs (Group 3)	58 (n/a)	8 (13.8)	5 (8.6)	3 (5.2)	0
Blood cultures (Group 4)	64 (n/a)	14 (21.9)	8 (12.5)	4 (6.3)	1 (1.6)

4.3.5 Description of disease characteristics of skin and soft tissue infections

Characteristics of community skin and soft tissue infections were taken from the information given on the microbiology request form by the requester. There was no information available for 2 specimens. 8 specimens (13.8%) were MRSA positive with the same number having toxin gene positivity. No specimens were both MRSA positive and toxin gene positive (see appendix). There was a wide variation in the source of infection. 18 specimens (31%) were from a wound or site of medical intervention, for example, a chest drain. This suggests that a proportion of these patients had recently been inpatients in a hospital. This is a potential major confounding variable. Two of these patients were toxin gene positive and four were MRSA positive.

4.3.6 Deprivation indices

There was no significant difference in deprivation index between those colonised and those infected with *S. aureus* (p=0.41) or between those who were colonised with *S. aureus* compared to those not colonised with *S. aureus* (p=0.99) as illustrated by figures 4-5 and

4-6. The Scottish Index of Multiple Deprivations identifies areas of deprivation within Scotland using postcodes. The Scottish Index of Multiple Deprivation 2009 combines 38 indicators across 7 domains, namely: income, employment, health, education, skills and training, housing, geographic access and crime.

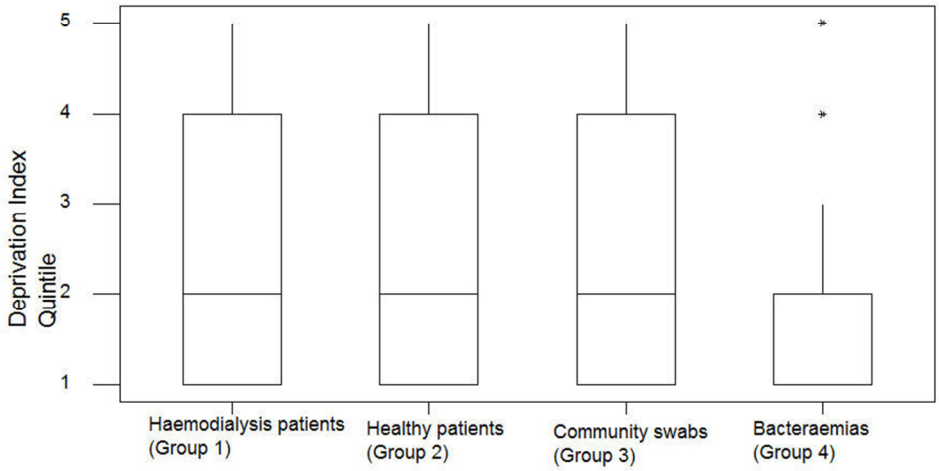


Figure 4-5 Boxplot of *S. aureus* positivity by Deprivation Index Quintile

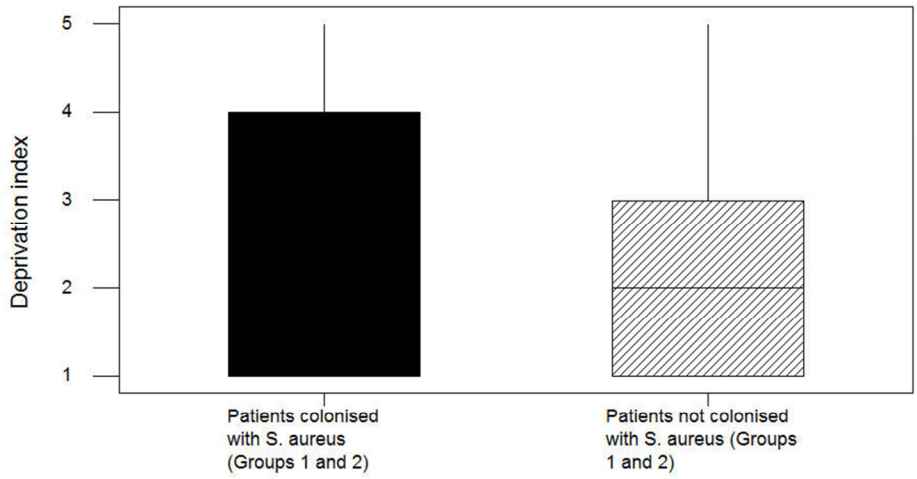


Figure 4-6 Boxplot of *S. aureus* colonisation compared to no *S. aureus* colonisation by deprivation index quintile

4.3.7 Spa testing and BURP diagrams

Spa testing was performed on all *S. aureus* positive isolates. Based Upon Repeat Pattern (BURP) diagrams were created for all positive isolates, for the individual groups and for toxin gene positive isolates. The spacing between linked *spa* types and between unlinked

spa types provides no information concerning the genetic distance between them. The *spa* type with the highest founder-score is defined founder of the cluster (blue colour). Subfounders are the *spa* types with the second highest founder-score and are labelled in yellow. If two or more *spa* types exhibit the same highest founder-score, they are all coloured in blue.

Table 4-7 Summary overview of numbers of isolated of MSSA, MRSA and number of *spa* types by study group

	No. isolates	**No. MSSA**	**No. MRSA**	**No. *spa* types MSSA**	**No. *spa* types MRSA**	**No. not typable**	**% not typable**
Group 1 Haemodialysis patients	44	40	4	34	4	0	0
Group 2 Healthy patients	34	33	1	28	1	0	0
Group 3 Community infections	58	50	8	35	6	2	4
Group 4 Bacteraemias	64	50	14	30	9	4	6.3

The diversity of the *spa* types can be quantified using Simpson's index of diversity (125) (245). MSSA only was included and the data were combined to represent isolates from colonised (Groups 1 and 2) or infected patients (Groups 3 and 4). Diversity was greater in the colonised groups compared to the infected patients however this did not reach statistical significance.

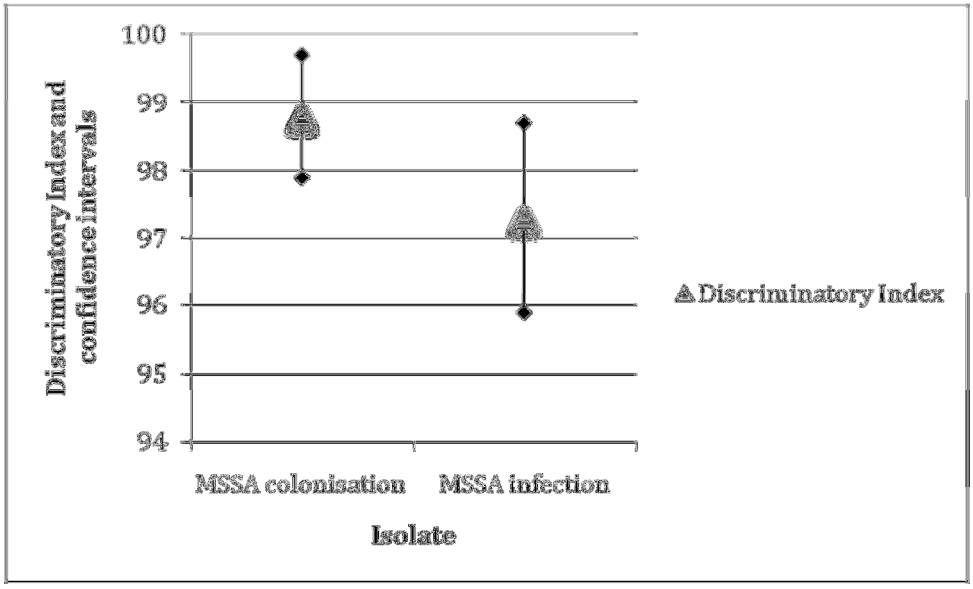


Figure 4-7 Estimates of genetic diversity expressed as Simpson's index of diversity of *spa* types (as a percentage) for MSSA of colonised patients (groups 1 and 2) and infected patients (groups 3 and 4)

BURP analysis of all isolates revealed 13 *spa* clusters and 17 singletons in total. This is illustrated using the population snapshot below.

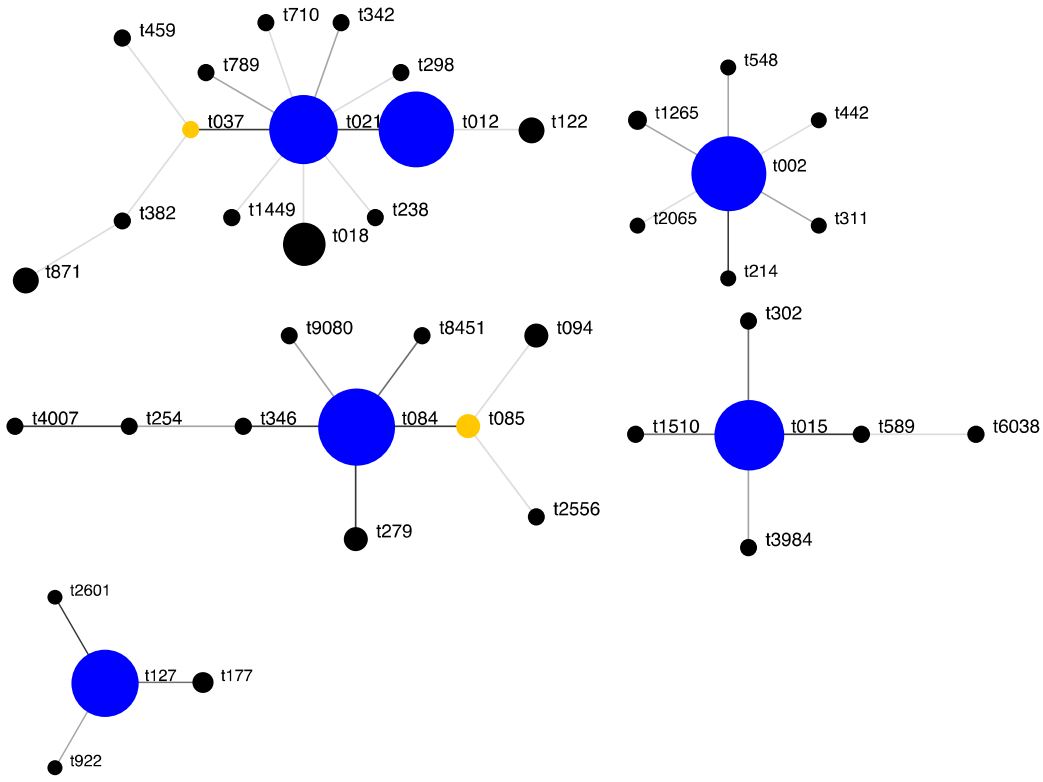


Figure 4-8 Population snapshot of all *S. aureus* strains on analysis of all isolates after BURP grouping. The *spa* type with the highest founder-score is defined founder of the

cluster (blue colour). Subfounders are the *spa* types with the second highest founder-score and are labelled in yellow

Isolates were grouped into MSSA isolates from patients colonised (Groups 1 and 2) and patients infected (Groups 3 and 4) with *S. aureus*. BURP analysis of the MSSA isolates from Groups 1 and 2 revealed 7 spa clusters and 19 singletons with the largest 2 associated with t012 and t015 (Figure 4-9). BURP analysis of the MSSA isolates from Groups 3 and 4 revealed 10 spa clusters and 13 singletons with the largest 2 associated with t032 and t012 (Figure 4-10).

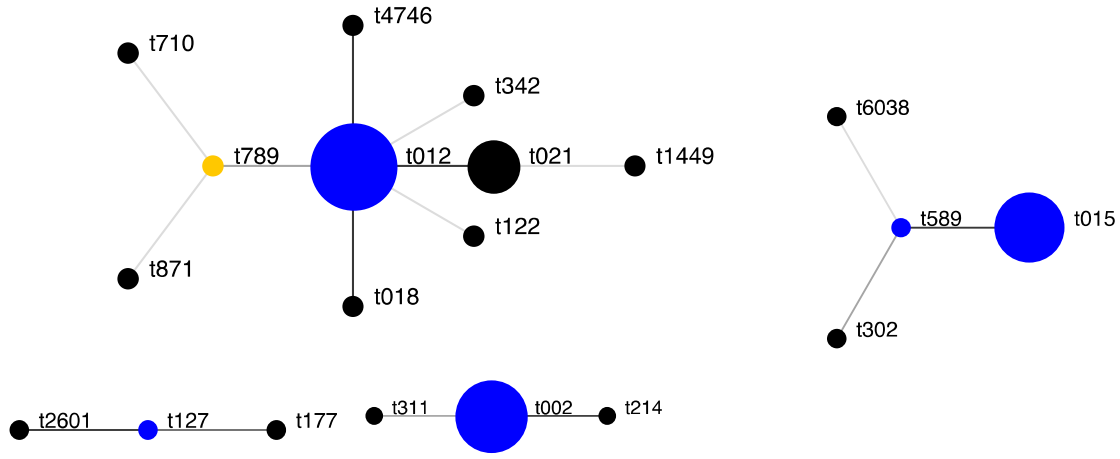


Figure 4-9 Population snapshot of the largest 4 clusters of MSSA isolates from Groups 1 and 2 after BURP grouping. The *spa* type with the highest founder-score is defined founder of the cluster (blue colour). Subfounders are the *spa* types with the second highest founder-score and are labelled in yellow

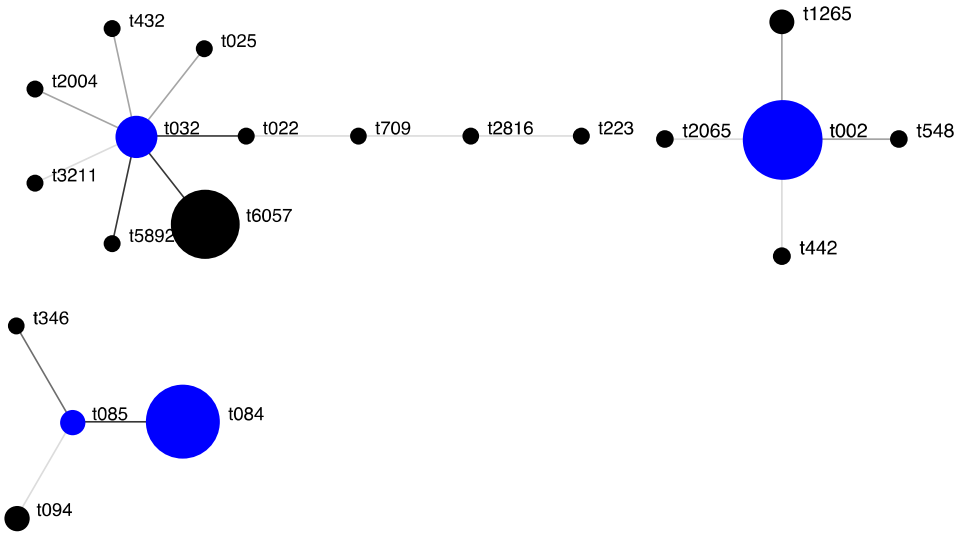


Figure 4-10 Population snapshot of the largest 2 clusters of MSSA isolates from Groups 3 and 4 after BURP grouping. The *spa* type with the highest founder-score is defined founder of the cluster (blue colour). Subfounders are the *spa* types with the second highest founder-score and are labelled in yellow

4.4 Discussion

Prevalence of *S. aureus* colonisation from this study was in keeping with previous research for haemodialysis patients and the general population (246) although it did not show a significantly higher point prevalence of *S. aureus* colonisation in haemodialysis patients. Overall, virulence toxin gene prevalence was low, accounting for 15.0% of all positive isolates and 7.6% of all isolates in total. This is similar to existing data for *S. aureus* bloodstream infections in Europe. Published data on *S. aureus* toxin gene positivity of colonised patients is sparse. There was no evidence of increased prevalence of toxin gene positivity if the isolates were grouped into those originating from isolated compared to infected patients (p=0.51).

These data suggest that although the virulence toxin genes may cause more severe disease, they are not more likely to cause disease in a colonised patient. Observational data from elsewhere in the UK suggests that the percentage of MRSA colonisation with PVL toxin gene positivity is increasing although MRSA colonisation in general is reducing (228). There appears to be significant geographical variation (229). There is also a potential difference in the age of those more likely to experience disease secondary to PVL positive *S. aureus* with paediatric patients more likely to develop the characteristic severe necrotising pneumonia associated with PVL positive *S. aureus* infection. This may partly

explain the absence of PVL positive *S. aureus* in this study as those under 18 were specifically excluded.

There is an ongoing national surveillance study funded by the Department of Health being performed by the Health Protection Agency focusing on patients presenting to English Emergency Departments or "Walk-in" centres with purulent skin infections (247). It is hoped that this will provide more information on the prevalence of PVL positivity in the community. Clearly, this is limited by the exclusion of isolates originating from General Practice, which would provide the majority of community *S. aureus* specimens.

Spa types of MSSA positive isolates representing infection (Groups 3 and 4) were predominately t002, t127 and t084 which were 1st, 2nd and 6th most frequent *spa* types isolated in a recent large European epidemiological study (248). t6057 was the predominant MRSA strain. This isolate is found mostly in Germany and the Netherlands (209). There was less epidemiological variation in the MRSA positive isolates than has been noted in previous research. Only countries for which *spa* type information for more then ten MRSA isolates were available were included in this figure (Figure 4-11).

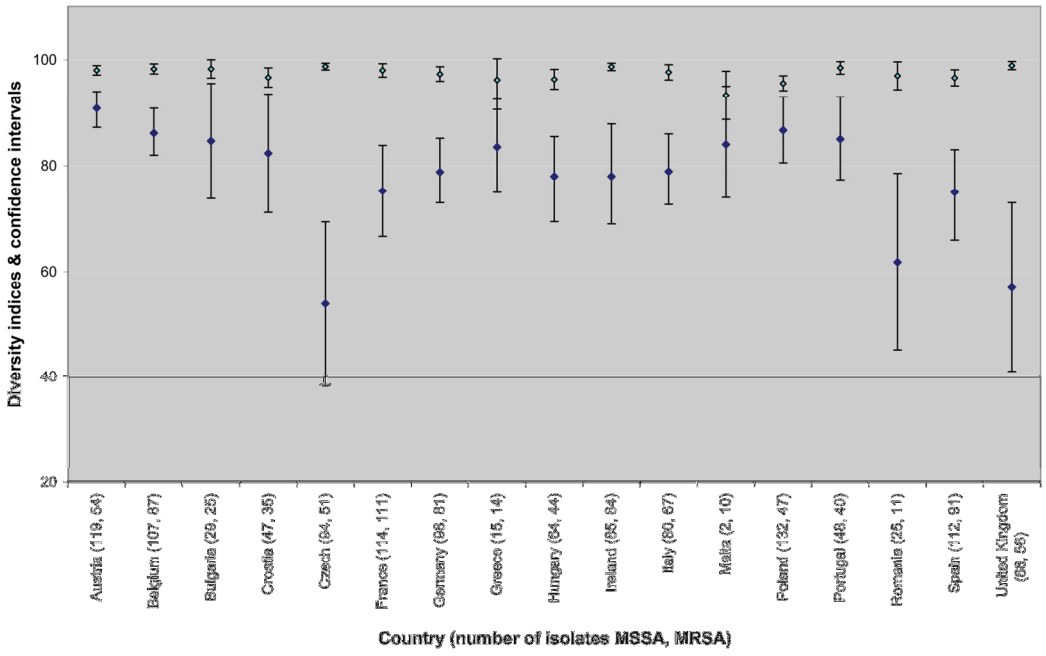


Figure 4-11 Estimates of country-specific genetic diversity expressed as Simpson's index of diversity of *spa* types (as a percentage) for MSSA (light blue diamonds) and MRSA (dark blue diamonds) and 95% CIs (bars)

The role of *S. aureus* colonisation in the development of systemic disease is unclear. There is an association between community based *S. aureus* skin infections and nasal carriage (249), however a large prospective population based study using all cause mortality as a surrogate end point for significant *S. aureus* disease found no association between persistent *S. aureus* nasal carriage and death (78). There is also evidence that those who are not colonised with *S. aureus* have an increased mortality rate if they do develop *S. aureus* bacteraemia compared to those with persistent nasal carriage (75). There is some evidence that *S. aureus* decolonisation using nasal mupirocin could reduce *S. aureus* infection in haemodialysis patients although repeated courses are required. There is concern that mupirocin use could promote an increased prevalence of high-level mupirocin resistance (250). There were only 4 cases of high-level mupirocin resistance in this dataset with 3 of these isolates being MRSA. None of these isolates were from haemodialysis patients.

The role of toxin gene positivity in *S. aureus* virulence has not been fully characterised and is felt to be part of a complex interplay between regulation of these and other virulence factors with the host response and factors influencing bacterial transmission. There is ongoing research into a vaccine against *S. aureus* and there is evidence of partial efficacy in haemodialysis patients in addition to healthy populations (251).

4.5 Conclusion

These data have demonstrated a similar prevalence of *S. aureus* colonisation in healthy patients and those undergoing hospital haemodialysis. They have added to existing evidence of a low prevalence of toxin gene positivity in the general population and haemodialysis patients in particular. There was no convincing evidence of increased virulence associated with toxin gene positivity manifest as skin infection in the community or bacteraemia, although numbers involved were small. Further research is required into the prevalence of toxin gene positivity in the general population with regard to asymptomatic colonisation and symptomatic infection.

4.6 Suggestions for further research

Larger scale research is ongoing into the prevalence of toxin gene positivity in community skin infections, however the most exciting new direction is whole genome sequencing of *S.*

aureus bacteria. This method has the potential to revolutionise the investigation of *S. aureus* transmission with particular application to the investigation of hospital-based outbreaks.

This method of whole genome sequencing of S. aureus was utilised in an English secondary and tertiary referral hospital with a 24 cot special care baby unit(252). A potential outbreak of MRSA colonisation was identified. Whole genome sequencing helped to distinguish between isolates that were part of the outbreak and those that were not involved and most importantly, identify a potential source. It also raised the currently unanswered issue of whether screening of staff for MRSA could be indicated in some situations. The pilot study in question screened staff after consulting with senior clinicians and the infection control team. It is stated that informed consent was obtained, however the implications of a staff member being colonised with MRSA were not explicitly stated.

Chapter 5: Prospective randomised double blind study of efficacy of probiotic milk drink (Yakult) in reducing the incidence of antibiotic associated diarrhoea and *Clostridium difficile* diarrhoea

5.1 Introduction

In 2006 there were more than 4000 documented cases of *C. difficile* infection in Scotland and concern was raised by the identification of a more virulent strain - NAP1/027 – which was felt to give rise to a more serious infection (253). A double blind randomised study has shown that 100g (97ml) Actimel administered twice daily within 48 hours of starting antibiotics and continued for 7 days after antibiotics were stopped reduced AAD and *C. difficile* in the treatment group (181). It found a 22% risk reduction for antibiotic associated diarrhoea (AAD) in the probiotic treated group (NNT = 5) and 17% reduction in *C. difficile* infection in the probiotic treated group (NNT= 6)(181). The positive results of this study are supportive of the use of probiotic drinks to reduce AAD and CDAD but require confirmation.

AAD and in particular *Clostridium difficile* associated diarrhoea (CDAD) are major causes of morbidity and mortality in hospitalised patients who are administered broad spectrum antibiotics. Cephalosporins, co-amoxiclav and quinolones are the main culprit antibiotics and the incidence of AAD may approach 25% of patients who are administered these antibiotics (254) (255).

C. difficile is found in up to 5% of the general population (151) (256) and in up to 18% of hospital inpatients (257). Its growth is restricted by normal bowel flora and it flourishes in the presence of antibiotic treatment. Its heat resistant spores can remain in the hospital environment for lengthy periods (258), are resistant to gastric acid (259) and alcohol based cleansing solutions (260) and this allows ready spread by the faecal-oral route. There is also evidence that spores can be disseminated via the aerial route (261).

Diarrhoea has been defined by the WHO as the new onset of 3 or more semi-formed or watery stools per 24 hours. The main predisposing factors to AAD in addition to exposure to antibiotics are age over 65 years, prolonged hospital stay and proton pump inhibitor therapy (161) (162). AAD usually occurs within 5-10 days of starting antibiotics but may develop earlier than 5 days or later than 10 weeks after stopping antibiotics (262) (263).

Probiotics are defined as live micro-organisms which confer a health benefit to the host when administered in adequate numbers (132). There is some evidence that probiotics may be helpful in reducing the incidence of AAD and *C. difficile* infections.

Further studies are required in high risk clinical settings, such as renal units, where broad spectrum antibiotics are frequently commenced on admission to hospital and the incidence rates of AAD and CDAD have been high.

5.2 Methods

5.2.1 Clinical setting

The renal inpatient wards are a high-risk clinical setting for AAD and CDAD. Patient demographics and diagnoses on admission were recorded and used as baseline characteristics for patient randomisation and study analyses. A baseline stool sample was sent to the microbiology laboratory and stored frozen for retrospective analysis for *C. difficile*. If CDAD developed in any patient, the baseline stool sample was retrospectively analysed using an alcohol-shock method followed by isolation on selective agar. Randomisation was stratified by age as this is a major risk factor for AAD with patients grouped by those aged younger than 65 or 65 and older at the time of randomisation.

5.2.2 Subjects

A. Inclusion criteria

1. Inpatient in a renal ward and prescribed a course of antibiotic with the exception of vancomycin alone or metronidazole alone or in combination with another antibiotic
2. aged 18 years or greater

B. Exclusion criteria

1. diarrhoea on randomisation or within the preceding week
2. lactose intolerance or intolerance to dairy products
3. regular probiotic use in the 4 weeks prior to randomisation
4. antibiotic use in the 4 weeks prior to randomisation
5. patients unable to give written consent within 48 hours of starting antibiotics

6. patients on induction dose immunosuppression (e.g. recent transplant or induction therapy of vasculitis)
7. active inflammatory bowel disease or bowel surgery less than 6 months prior to admission

All patients were managed as per the hospital infection control recommendations to minimise the risk of developing or transferring infectious diarrhoea, for example, gowns and gloves and isolation rooms as deemed appropriate by the supervising clinician. The principal stopping criterion was the development of lactobacillus bacteraemia in any patient suggesting systemic infection with the probiotic strain.

5.2.3 Study protocol

Patients commencing antibiotics were asked for informed consent within 48 hours of starting antibiotics. Patients were randomised to either probiotic milk drink (Yakult) twice daily or placebo solution twice daily within 48 hours of starting one or more of the above antibiotics and continued until 7 days after stopping antibiotics. Patients, nursing staff and investigators were blinded to which study product had been assigned to the patient.

Randomisation was via an online portal designed by the Robertson Centre of Biostatistics, University of Glasgow. Compliance with the randomised non-medicinal product (Yakult or placebo) was supervised by the renal unit medical and nursing staff.

Stool samples were sent for culture to attempt to identify a specific cause in all patients who developed diarrhoea as per usual practice. Cell culture method was used to diagnose the presence of CDAD. The frequency and duration of diarrhoea, identified aetiology (if any), and antimicrobial treatment of diarrhoea (if any), were recorded. Antimicrobial treatment adhered to local hospital guidelines on antibiotic treatment for *Clostridium difficile* and other enteric infections such as shigella and campylobacter.

Patients were followed up for 12 weeks after recruitment with AAD, CDAD, further hospital admission and mortality documented.

The milk drinks were checked on arrival and storage refrigerators were checked regularly to ensure the desired temperature range was adhered to. If the drinks were found to be too warm they were discarded.

5.2.4 Study outcomes

A. Primary outcome
Incidence of AAD:
AAD was assessed by daily monitoring of frequency of loose or semi-formed stools by renal nursing staff and development of abdominal pain. Diarrhoea was defined as 3 or more loose bowel motions in 24 hours according to the World Health Organisation definition. Stool samples were sent following the diagnosis of AAD.

B. Main secondary outcome
Incidence of *C. difficile* diarrhoea:
C. difficile cell culture and stool cultures were performed on all patients with undiagnosed diarrhoea to identify if enteric pathogens were present.

C. Other secondary outcomes

1. total days until discharge from hospital or patient death
2. total treatment days on oral metronidazole or vancomycin
3. adverse events attributable to Yakult or control solution
4. significant differences in routine laboratory tests such as serum albumin

5.2.5 Statistical analyses

Data were analysed using an intention to treat analysis. Chi squared test, Mann Whitney U test and Student's unpaired T test were used where appropriate.

5.2.6 Power calculation

With α=0.05 and a power of 90% to detect an absolute difference of 20% between the proportion of patients with antibiotic associated diarrhoea in the placebo (assumed at 30%) and probiotic (assumed at 10%) groups we estimated that we needed a sample size of 164 (82 in each group).

5.2.7 Indemnity and ethical approval

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and was healthcare related research covered by NHS indemnity. It was approved by the local Research and Development department and by NHS Greater Glasgow and Clyde research and ethics committee, (reference 08/S0704/4), on the 11th July 2008.

5.3 Results

During a 44 month period (01/03/2009 – 01/11/2012) 85 patients, who were admitted to the renal unit and met the inclusion and exclusion criteria, were recruited to this study within 2 days of starting antibiotics. Forty-four of the patients were randomised to probiotic milk drink and 41 to placebo. Seven hundred and sixteen additional patients were screened.

The main reasons why patients were not recruited were:

- recent antibiotic use (n=220)
- current metronidazole use (n=75)
- declined to take part in the study (n=68)
- antibiotic administration for >48 hours (n=65)
- unable to give informed consent (n=59)
- vancomycin prescribed as a single agent (n=51)
- too unwell to participate (n=43)
- diarrhoea (n=43)
- long term antibiotic use (n=37)
- high dose immunosuppression (n=36)
- unknown (n=19)

Figure 5-1 is a consort diagram illustrating the recruitment process of patients screened to take part in the study.

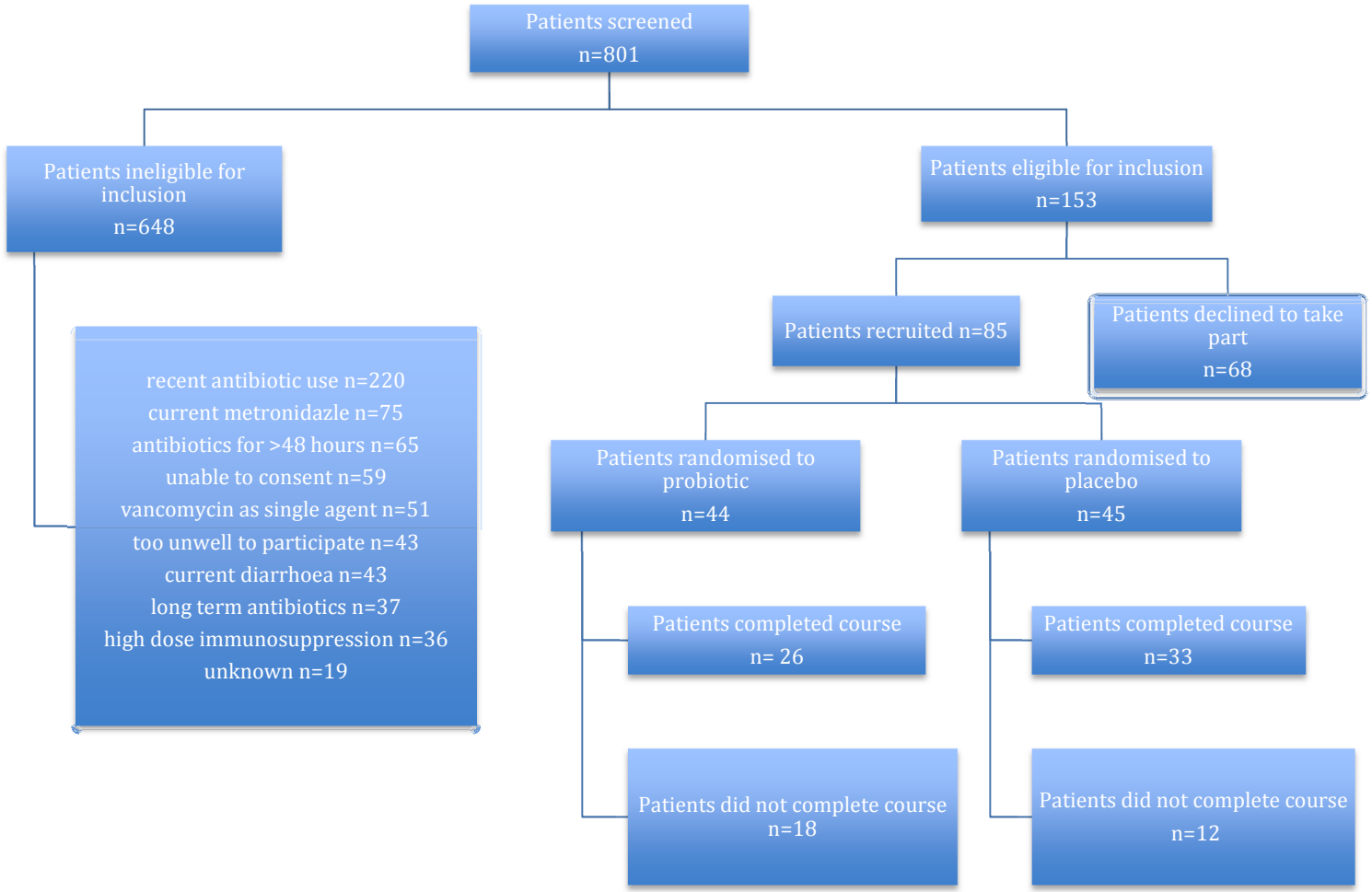


Figure 5-1 Consort diagram of the recruitment process of patients screened to take part in the probiotics study

Table 5-1 Patient characteristics at recruitment

Characteristic	Probiotic milk drink (n=44)	Placebo (n=41)	P value
Age (mean, IQR*)	62.27 (52.02, 74.43)	62.49 (50.69, 76.40)	0.949
Sex (% male)	27 (61.4)	26 (63.4)	0.845
Renal replacement therapy			
emergency haemodialysis	2	4	0.352
longterm haemodialysis	25	20	0.457
peritoneal dialysis	4	5	0.643
renal transplant	3	3	0.929
Antibiotic duration (mean, IQR)	13.75 (7.0, 14.0)	11.40 (6.0, 10.75)	0.545
Comorbidity			
diabetes mellitus	15 (34.09)	15 (36.59)	0.810
ischaemic heart disease	16 (36.36)	16 (39.02)	0.800
cerebrovascular disease	7 (15.91)	7 (17.07)	0.885
hypertension	29 (65.91)	32 (78.05)	0.208
asthma/COPD**	5 (11.36)	8 (19.51)	0.298
malignancy	2 (4.55)	4 (9.76)	0.352
vasculitis	0	2 (4.88)	0.147
Previous CDAD	1 (2.27)	2 (4.88)	0.520
Medications			
proton pump inhibitor	27 (61.36)	20 (48.78)	0.240
antidiarrhoeal agents	0	1 (2.44)	0.311
immunosuppression	8 (18.18)	6 (14.63)	0.658
Laboratory parameters			
haemoglobin(mean, IQR)	10.5 (9.1, 11.9)	10.3 (8.7, 12.0)	0.378
white cell count (mean, IQR)	9.36 (6.79, 10.99)	10.97 (8.05, 12.45)	0.057
platelet count (mean, IQR)	233 (154, 296)	233 (182, 280)	0.894
C reactive protein (mean, IQR)	109 (40, 163)	98 (17, 170)	0.555
Serum albumin	28.8 (26.3, 33.0)	28.0 (23.5, 32.0)	0.550

*interquartile range

** chronic obstructive pulmonary disease

Table 5-1 shows that there were no significant differences in patient baseline characteristics between the probiotic milk drink and placebo groups at the time of randomisation.

There was no significant difference in primary and secondary outcomes between those taking probiotic milk drink and placebo as illustrated by Table 5-2. The only exception to this was improved compliance in the placebo group at the end of the antibiotic course (85.4% v 65.9%; p=0.031). This difference was not maintained at completion of the study (p=0.123). AAD occurred in 36.36% of cases in the probiotic milk drink group and 34.14% in the placebo group. There was no significant difference when non compliant patients were excluded from the analysis. Only 2 patients developed CDAD during the study period. Both of the patients with CDAD were in the placebo group. Neither patient developed toxic megacolon or required colectomy. Both patients had baseline stool specimens submitted. One of these specimens grew *C. difficile* ribotype 002.

Nausea was the most common side-effect in both groups and was the predominant reason given for non compliance with the drinks. Elevated tacrolimus levels were noted in 1 patient who was in the placebo arm of the study.

There was no significant change in laboratory parameters between probiotic and placebo groups although the study was not powered for this.

47.7% patients in the probiotic group and 36.6% patients in the placebo group were readmitted to the renal unit during the 3 month follow up period (p=0.295). 13.3% and 12.2% patients died during the 3 month follow-up period in the probiotic and placebo groups respectively (p=0.843). This trend toward an increased rate of readmission may reflect an underlying increase in co-morbidity in the probiotic group that was not captured by the baseline characteristics recorded. There was no significant difference in length of admission between the two groups, including when the analyses were censored for inpatient deaths and for deaths during follow-up.

Table 5-2 Comparison of patient outcomes between probiotic milk drink and placebo groups

	Probiotic milk drink n=44 (%)	**Placebo n=41 (%)**	**P value**
Antibiotic associated diarrhoea	16 (36.36)	14 (34.14)	0.831
***C. difficile* diarrhoea**	0	2 (4.87)	0.147
Compliance			
2 days after starting	32 (72.73)	36 (87.80)	0.074
at end of antibiotic course	29 (65.91)	35 (85.37)	**0.031**
1 week after stopping antibiotic	28 (63.63)	33 (80.49)	0.077
Reasons for non compliance/ side effect			
nausea	7 (15.90)	3 (7.31)	0.210
change in immunosuppression levels	0	1 (2.44)	0.311
disruption in supply of drink	6 (13.63)	3 (7.31)	0.337
deterioration in patient condition	2 (4.54)	1 (2.44)	0.595
patient changed their mind regarding taking part in study	2 (4.54)	1 (2.44)	0.595
Antibiotic associated diarrhoea in fully compliant patients	9/26 (34.6%)	11/33 (33.3%)	0.918
Change in laboratory parameters between randomisation and discharge (death censored)			
Hb (g/dl) (mean, IQR)	-0.43 (-1.23, 0.13)	-0.56 (-1.18, 0.48)	0.654
WCC (mean, IQR)	-0.74 (-3.15, 0.85)	-2.24 (-3.93, 0.06)	0.089
Platelet count (mean, IQR)	37 (18, 75)	37 (-3, 64)	0.951
C reactive protein (mean, IQR)	-59 (-126, 0)	-55 (-131, 2)	0.855
Serum albumin (mean, IQR)	-1.9 (-4.0, 0.25)	-1.8, (-5.0, 1.0)	0.928
Length of hospital admission (mean, IQR)	10.16 (3, 12)	13.8 (6, 19)	0.104
Readmitted during 3 month follow-up	21 (47.7)	15 (36.6)	0.295
Died during hospital admission	2 (4.5)	1 (2.4)	0.595
Died during 3 month follow-up	6 (13.3)	5 (12.2)	0.843
Composite of AAD/ CDAD/ death	20 (45.5)	15 (36.7)	0.404

Table 5-3 compares antibiotic burden between probiotic and placebo groups by the antibiotic choice at recruitment. There was no significant difference in the proportion of patients taking 2 or more antibiotics at the time of recruitment. There were more patients prescribed flucloxacillin and gentamicin in the probiotic group. These data are potential confounders as there is good evidence that antibiotic choice is associated with the development of AAD including CDAD. It is notable that there was no difference between

the 2 groups in the proportion of patients prescribed high risk antibiotics such as co-amoxiclav, cephalosporins and fluroquinolones.

Table 5-3 Comparison of antibiotic choice at recruitment between probiotic and placebo groups

	Probiotic n=44 (%)	**Placebo n=41 (%)**	**P value**
Specific antibiotics			
amoxicillin	7 (15.9)	14 (33.3)	0.056
benzylpenicillin	-	1 (2.4)	0.311
ceftazidime	3 (6.8)	4 (9.5)	0.647
ciprofloxacin	10 (22.7)	8 (19.0)	0.674
clarithromycin	3 (6.8)	7 (16.7)	0.153
clindamycin	-	1 (2.4)	0.311
co-amoxiclav	3 (6.8)	3 (7.1)	0.953
doxycycline	-	1 (2.4)	0.311
flucloxacillin	10 (22.7)	3 (7.1)	**0.037**
gentamicin	14 (31.8)	6 (14.3)	**0.048**
levofloxacin	1 (2.3)	-	0.312
rifampicin	1 (2.3)	-	0.312
tazocin	2 (4.5)	3 (7.1)	0.608
teicoplanin	-	1 (2.4)	0.311
vancomycin	13 (29.5)	8 (19.0)	0.252
number of patients prescribed 2 or more antibiotics	20 (45.5)	20 (47.6)	0.759

Table 5-4 examines antibiotic use associated with AAD including CDAD. There was no significant difference in the incidence of AAD between the probiotic milk drink and placebo groups in those prescribed 2 or more antibiotics. There was more AAD in the placebo group in those prescribed flucloxacillin and gentamicin although caution is required in the interpretation of this given the small numbers involved and the tendency for these antibiotics to be prescribed concomitantly, particularly in the presence of gram positive infection. All antibiotics prescribed during the follow-up period are included in these data.

Table 5-4 Antibiotic associated diarrhoea including *C. difficile* associated diarrhoea by antibiotic

	Probiotic milk drink no. of patients with AAD (% of total taking named antibiotic)	**Placebo no. of patients with AAD (% of total taking named antibiotic)**	**P value**
amoxicillin	3 (30.0)	5 (27.8)	0.901
benzylpenicillin	0 (0)	1 (100)	n/a
ceftazidime	2 (66.7)	0	n/a
ciprofloxacin	4 (30.8)	4 (40.0)	0.646
clarithromycin	2 (66.7)	1 (14.3)	0.083
co-amoxiclav	2 (50.0)	2 (40.0)	0.764
flucloxacillin	3 (23.1)	3 (75.0)	**0.035**
gentamicin	4 (28.6)	5 (71.4)	**0.040**
levofloxacin	1 (100)	0 (0)	n/a
rifampicin	0 (0)	1 (100)	n/a
tazocin	2 (66.7)	3 (75.0)	0.811
vancomycin	5 (29.4)	3 (33.3)	0.838
number of patients with AAD prescribed 2 or more antibiotics	10 (66.7)	8 (57.1)	0.716

Table 5-5 Site of infection at recruitment

	Probiotic milk drink (n=44 patients)	Placebo (n=41 patients)	P value
blood	1	0	0.312
chest	9	13	0.235
endocarditis	0	1	0.311
line	7	3	0.210
PD* peritonitis	3	4	0.624
skin	10	5	0.195
unknown	6	10	0.843
urine	8	5	0.439

*peritoneal dialysis

Comparison of the site of infection between patients randomised to probiotic and patients randomised to placebo is illustrated in Table 5-5. There were no significant differences in the site of infection between the 2 groups at initiation of treatment.

Table 5-3 Antibiotic associated diarrhoea by site of infection

	Probiotic milk drink no. of patients with AAD (% total)	Placebo no. of patients with AAD (% total)	P value
blood	0 (0)	0 (0)	-
chest	3 (33.3)	3 (23.1)	0.600
endocarditis	0 (0)	1 (100)	-
line	3 (42.9)	1 (33.3)	0.773
PD* peritonitis	2 (66.7)	0 (0)	**0.014**
skin	3 (30.0)	0 (0)	**0.038**
unknown	2 (33.3)	5 (50.0)	0.503
urine	3 (37.5)	4 (80.0)	0.086

Table 5-6 illustrates the percentage of patients with AAD separated into site of primary infection. It suggests that those patients with PD associated peritonitis and randomised to probiotic milk were more likely to experience AAD but the number of cases was very small and there were no patients with PD peritonitis in the placebo group. This difference is not maintained with the composite end point of AAD, CDAD and death (Table 5-7).

Table 5-4 Antibiotic associated diarrhoea, CDAD or death by site of infection

	Probiotic milk drink no. of patients with AAD/CDAD or died (% total)	**Placebo** no. of patients with AAD/CDAD or died (% total)	P value
blood	0 (0)	0 (0)	
chest	5 (55.6)	3 (23.1)	0.109
endocarditis	0 (0)	1 (100)	-
line	4 (57.1)	1 (33.3)	0.471
pd peritonitis	3 (100)	0 (0)	-
skin	3 (30.0)	1 (20.0)	0.664
unknown	2 (33.3)	5 (50.0)	0.503
urine	4 (50.0)	4 (80.0)	0.233

Patients were stratified according to age (younger than 65 or 65 and older) at the time of randomization. When these groups are compared, there are no significant differences in outcome with the exception of increased compliance seen at the end of the antibiotic course in the placebo group aged younger than 65 as shown in Tables 5-8 and 5-9. However this higher compliance was not maintained at completion of the study 7 days after stopping antibiotics as was also noted when the patients younger than 65 or 65 and older were analysed as a single group in Table 5-2.

Table 5-5 Comparison of patient outcomes between probiotic milk drink and placebo groups in those aged younger than 65

	Probiotic milk drink n=24 (%)	**Placebo n=20 (%)**	**P value**
Antibiotic associated diarrhoea	8 (32.0)	8 (40.0)	0.648
***C. difficile* diarrhoea**	0	0	
Compliance			
2 days after starting	19 (76.0)	18 (90.0)	0.310
at end of antibiotic course	15(60.0)	18 (90.0)	**0.021**
1 week after stopping antibiotic	15 (60.0)	17 (85.0)	0.077
Reasons for non compliance/ side effect			
nausea	5 (20.0)	1 (5.0)	0.100
change in immunosuppression levels	0	1 (5.0)	0.305
disruption in supply of drink	4 (25.0)	0	**0.020**
deterioration in patient condition	0	1 (5.0)	0.305
patient changed mind regarding taking part in study	0	1	0.305
Antibiotic associated diarrhoea in fully compliant patients	3/15 (20.0%)	4/17 (23.53%)	0.809
Readmitted during 3 month follow-up	10 (40.0)	8 (40.0)	1.000
Died during hospital admission	0	0	
Died during 3 month follow-up	3 (12.0)	1 (5.0)	0.389
Composite of AAD/ CDAD/ death	10 (40.0)	8 (40.0)	1.000

Table 5-6 Comparison of patient outcomes between probiotic milk drink and placebo groups in those aged 65 and older

	Probiotic milk drink n=20 (%)	**Placebo n= 21 (%)**	**P value**
Antibiotic associated diarrhoea	8 (40.0)	6 (28.6)	0.438
***C. difficile* diarrhoea**	0	2 (9.5)	0.137
Compliance			
2 days after starting	13 (65.0)	18 (85.7)	0.114
at end of antibiotic course	13 (65.0)	17 (81.0)	0.412
1 week after stopping antibiotic	12 (60.0)	16 (76.2)	
Reasons for non compliance/ side effect			
nausea	2 (10.0)	2 (9.5)	0.959
change in immunosuppression levels	0	0	-
disruption in supply of drink	2 (10.0)	2 (9.5)	0.959
deterioration in patient condition	2 (10.0)	1 (4.8)	0.972
patient changed mind regarding taking part in study	2 (10.0)	0	0.136
Antibiotic associated diarrhoea in fully compliant patients	4/12	4/16	0.632
Readmitted during 3 month follow-up	10 (50.0)	7 (33.3)	0.273
Died during hospital admission	1 (5.0)	1 (4.8)	0.972
Died during 3 month follow-up	2 (10.0)	3 (14.3)	0.673
Composite of AAD/ CDAD/ death	11 (55.0)	7 (33.3)	0.153

Univariate binomial regression analysis was performed in order to determine factors associated with the development of AAD. Univariate regression analysis is shown in Table 5-10. No variable gave a statistically significant result suggesting either an increased or decreased risk of AAD.

Table 5-7 Univariate analysis of factors associated with antibiotic associated diarrhoea

Variable	Odds ratio	Lower CI	Upper CI	P value
age	1.01	0.98	1.04	0.647
sex	1.68	0.65	4.32	0.279
2 or more antibiotics	1.33	0.53	3.38	0.543
total antibiotics days	1.03	0.99	1.07	0.102
AKI	1.13	0.36	3.47	0.838
Acute on CKD*	2.04	0.60	7.00	0.259
CKD* not on RRT**	1.41	0.55	3.62	0.476
RRT**	0.53	0.20	1.36	0.185
Diabetes mellitus	2.13	0.85	5.37	0.107
IHD¥	1.17	0.47	2.91	0.741
Cerebrovascular disease	0.69	0.20	2.43	0.559
Hypertension	0.53	0.20	1.41	0.207
Asthma/COPD¥¥	1.18	0.35	3.97	0.796
Malignancy	0.91	0.16	5.29	0.917
Vasculitis	1.86	0.11	30.88	0.667
Immunosuppressed	1.02	0.31	3.38	0.971
Previous CDAD	3.86	0.34	44.41	0.261
PPI use	2.07	0.82	5.23	0.117
Antidiarrhoeals	0.00	0.00	-	0.356
Laxatives	0.72	0.25	2.03	0.526
Further admission	1.06	0.43	2.61	0.893
Died during follow-up period	1.63	0.45	5.88	0.456

* Chronic kidney disease

** Renal replacement therapy

¥ Ischaemic heart disease

¥¥ Chronic obstructive pulmonary disease

The univariate analysis was repeated using a composite end point of AAD, CDAD and death as illustrated in Table 5-11. Proton pump inhibitor (PPI) use was the only variable identified to be significantly associated with an increased risk of AAD, CDAD and death with an odds ratio of 2.79 (P= 0.23). Total number of days on antibiotics was also associated with increased risk with odds ratio 1.05 (P=0.017). Requiring long-term RRT appeared to be protective with odds ratio 0.42 although this did not reach statistical significance (p= 0.066).

Table 5-8 Univariate analysis of factors associated with antibiotic associated diarrhoea, CDAD and death

Variable	Odds ratio	Lower CI	Upper CI	P value
age	1.02	0.99	1.05	0.183
sex	1.12	0.46	2.73	0.802
2 or more antibiotics	1.12	0.46	2.73	0.802
total antibiotics days	1.05	1.00	1.11	**0.017**
AKI	2.00	0.67	6.01	0.214
Acute on CKD	2.12	0.61	7.34	0.230
CKD not on RRT	1.41	0.56	3.55	0.462
RRT	0.42	0.16	1.07	**0.066**
Diabetes mellitus	2.00	0.81	4.94	0.131
IHD	1.34	0.55	3.26	0.513
Cerebrovascular disease	1.45	0.46	4.57	0.528
Hypertension	0.82	0.32	2.13	0.684
Asthma/COPD	0.83	0.25	2.77	0.757
Malignancy	0.66	0.11	3.83	0.639
Vasculitis	1.37	0.08	22.69	0.826
Immunosuppressed	1.02	0.32	3.26	0.967
Previous CDAD	2.82	0.25	32.41	0.388
PPI use	2.79	1.13	6.90	**0.023**
Antidiarrhoeal agents	0.00	0.00	-	0.292
Laxatives	0.76	0.28	2.04	0.579
Further admission	0.95	0.40	2.28	0.913

Multivariate binomial regression analysis for a composite of AAD, CDAD and death was performed using a backwards selection method for those with p values < 0.2 (Table 5-12). Patients requiring renal replacement therapy appear to have reduced risk of AAD, CDAD and death as a composite end point (OR 0.33, P= 0.054). PPI use was associated with significantly increased risk of meeting the composite end point with OR 2.64. (P= 0.003).

Table 5-9 Multivariate analysis of factors associated with antibiotic associated diarrhoea, CDAD and death

Variable	Univariate analysis			Multivariate analysis		
	Odds ratio	**Confidence Interval**	**P value**	**Odds ratio**	**Confidence Interval**	**P value**
Age	1.02	0.99, 1.05	0.183			ns
Diabetes mellitus	2.00	0.81, 4.94	0.131			ns
Total antibiotic days	1.05	1.00, 1.11	1.05			ns
RRT	0.42	0.16, 1.07	0.066	0.33	0.12, 0.92	0.054
PPI	2.79	1.13, 6.90	0.023	2.64	0.99, 7.03	0.003

5.4 Discussion

This double blind, randomized placebo controlled study of high risk renal inpatients has shown no evidence of a decreased incidence of AAD or CDAD when prescribed a commercially available probiotic milk drink twice daily within 2 days of starting a course of antibiotics (Table 5-2). However, there was a much lower incidence of CDAD in the current study than in previously reported studies and the Cochrane Review. Only 2 (2.4%) of the 84 patients recruited to this study developed symptomatic CDAD, whereas there was a high incidence of AAD in both the probiotic milk drink and placebo groups. The Cochrane Systematic Review found a much higher CDAD incidence of 12.6% in the probiotic groups and 12.7% in placebo groups in 13 studies (n=961) (183). The WHO definition of diarrhoea was chosen to give a formal definition to be used by clinicians, however, it is limited by the lack of clarity in what constitutes "loose stool".

Clostridium difficile cases in Scotland were falling consistently throughout the duration of the study from 1.29 per 1000 occupied bed days in 2007-8 to 0.296 per 1000 occupied bed days in 2011-12. Cases of CDAD in the Glasgow Renal Units also fell between 2008 and 2013 with 43 cases in 2008 compared to 8 in 2012 with no significant difference in the total number of renal inpatient beds or bed occupancy rates. The results of this study therefore need to be interpreted in the knowledge that the study was performed during a period of time when a bundle of measures was introduced to reduce the incidence of CDAD to rates much below the incidence reported in previous clinical trials.

A range of preventive measures for CDAD was instituted locally and nationally during the study time period after a well publicised outbreak of CDAD infection and associated increase in mortality was reported in a specific hospital in NHS Greater Glasgow and Clyde. Firstly, antibiotic guidelines were changed to restrict the use of high-risk antibiotics, in particular, co-amoxiclav and cephalosporins, and the subsequent reduction in the use of antibiotics at higher risk of inducing CDAD is shown in the data below provided by the Greater Glasgow and Clyde Antimicrobial Prescribing Group (Figure 5-2). Defined daily dose (DDD) of cephalosporins per 1000 occupied bed days in NHS GGC fell from a peak of 78 between April and June 2008 to 30 between July and September 2009. Co-amoxiclav use also halved over a similar time period.

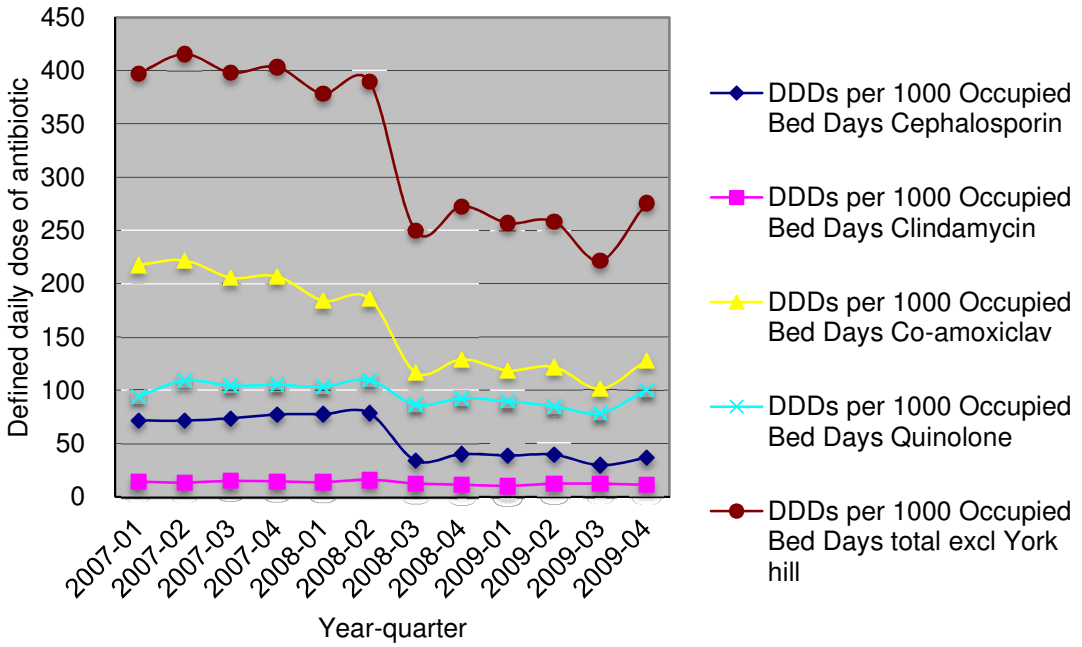


Figure 5-2 Antibiotic use before and after change in empirical antibiotic guidelines in August 2008

Antibiotic protocols for NHS Greater Glasgow and Clyde before and after the changes instituted to reduce CDAD are illustrated in Figures 5-4 and 5-5 below. It is not always possible to adhere to empirical antibiotic guidelines and avoid the use of antibiotics at high risk of CDAD in the renal unit population. For example trimethoprim or gentamicin may need to be avoided due to the risks of increasing serum potassium and/or serum creatinine concentrations in patients with severe renal impairment (264) and there is a risk of drug interactions in those patients taking immunosuppressive medications and antibiotics such as rifampicin and clarithromycin.

Secondly, an infection control nursing care plan was put in place for patients with loose stool of unknown origin. It covered multiple aspects of care including appropriate accommodation for patients with diarrhoea, attention to hand hygiene, personal protective equipment, decontamination of patient equipment, appropriate collection of stool specimens, laundry, waste disposal, environmental cleaning, and toileting facilities. The care plan for patients with confirmed CDAD also stressed the requirement for appropriate hand hygiene and reinforced that alcohol gel was ineffective against CDAD spores. It also stated that patients should not be transferred to other hospital wards or departments with confirmed CDAD until they were 48 hours free of symptoms and have had a formed stool.

With this extensive bundle of preventive measures the targets for a reduction in CDAD incidence rates were achieved as shown by Figure 5-3 below. For the year ending March 2013, the CDAD rate across NHS Scotland was 0.37 per 1,000 occupied bed days among patients aged 65 and over. The current HEAT target is to reduce CDAD cases to 0.32 cases or less per 1,000 total occupied bed days by March 2015.

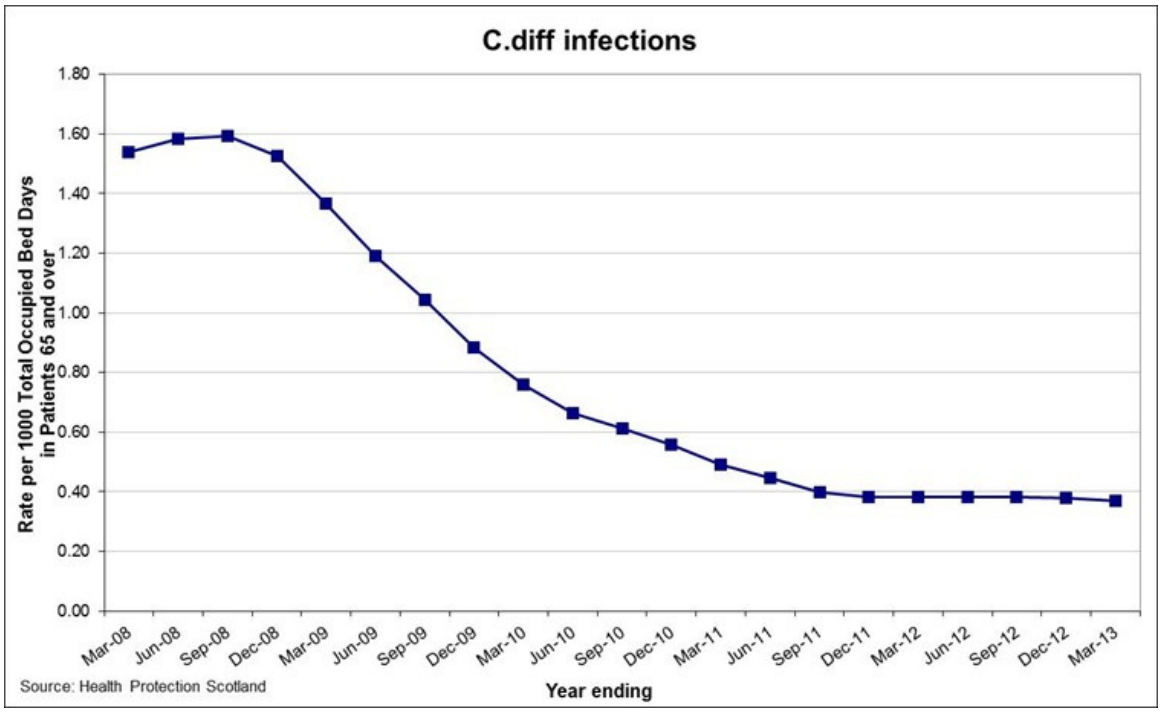


Figure 5-3 National performance for CDAD cases March 2008- March 2013

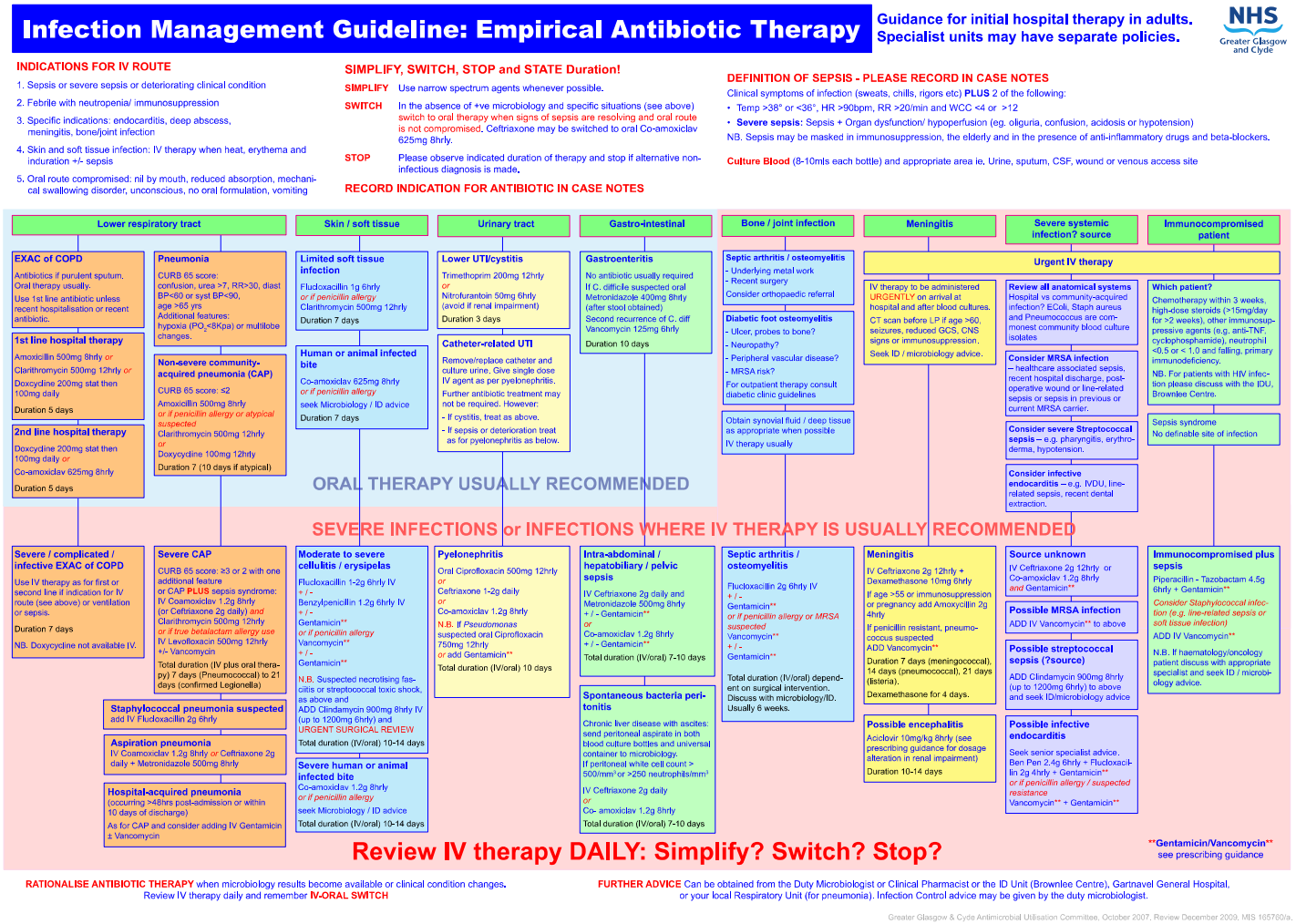


Figure 5-4 Empirical antibiotic guidelines in Greater Glasgow and Clyde pre June 2008

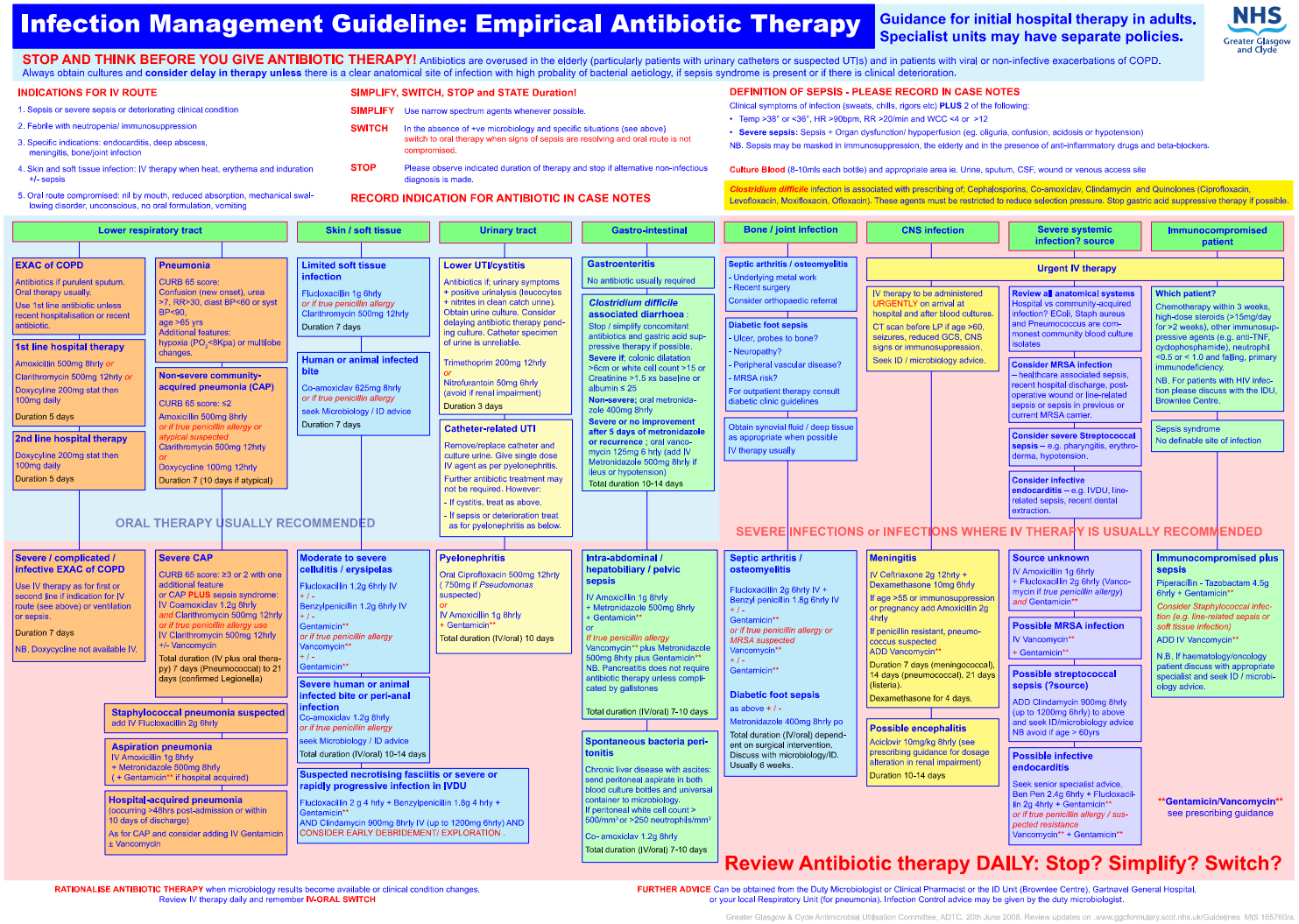


Figure 5-3 Empirical antibiotic guidelines in Greater Glasgow and Clyde post June 2008

Recruitment to this study did not meet the total number required from the power calculation but there was not even a trend towards a reduction in AAD in the probiotic group. Indeed there was a 2% increase in AAD in the probiotic group compared with the placebo group. The low incidence rates of CDAD observed in both groups in the current study were most likely related to a bundle of preventive measures introduced in all hospital patients during the study period. As a consequence of the falling background CDAD rate during the study period, the initial power calculation was no longer valid. Recruitment was slower than projected due to a number of factors including delays in transferring patients to the tertiary unit resulting in antibiotic use for longer than 48 hours prior to admission to the renal unit and higher than expected community antibiotic use.

There is some recent evidence from the USA that a higher cumulative antibiotic dose is associated with an increased risk of CDAD. Stevens *et al* conducted a retrospective cohort study of over 10 000 hospital admissions and found that cumulative dose, number and duration of antibiotic use were associated with an increased risk of the development of CDAD across all classes of antibiotics although those taking cephalosporins, β-lactamase inhibitor combinations, fluoroquinolones, sulphonamides, and intravenous vancomycin were significantly more likely to develop CDAD compared with patients who did not receive these antibiotics, regardless of any other antibiotics that were prescribed. Those patients taking fluoroquinolones were at the highest risk (265). In a paediatric population co-amoxiclav was more frequently associated with the development of AAD than other antibiotics (266). This study highlighted that it is important to recognize the patient burden associated with AAD as well as CDAD.

The recent Cochrane Systematic Review evaluated the effect of probiotics on prevention of AAD and CDAD and identified 23 randomized controlled trials reporting on a total of 4213 patients (183). It found that the incidence of CDAD was 2.0% in the probiotic group compared to 5.5% in the placebo or no treatment control group with a relative risk of 0.36 (95% CI 0.26 to 0.51) and rated the quality of this evidence as moderate. Evidence for the effect of probiotics on AAD was found to be less robust and rated as low quality. In twenty-five studies with a total of 4097 patients 13% of the participants in the probiotics group developed AAD compared to 21% of the placebo or no treatment control groups with a relative risk of 0.60 (95% CI 0.49 to 0.72). The studies on AAD were heterogenous with a high proportion of missing data and, if this was taken into account, there was no longer a statistically significant reduction in AAD in those taking a probiotic (RR 0.90;

95% CI 0.69 to 1.18).

Data in press or published recently continue to provide support for the concurrent use of probiotics in prevention of AAD and CDAD. A *Lactobacillus casei* Shirota (*Lc*S) preparation was used in a randomized study in a total of 164 patients with spinal cord injuries within 24 hours of starting antibiotics (267). The incidence of AAD in the *Lc*S group was 17.1% compared with 54.8% in the control group ($p<0.001$). Patients at risk of undernutrition were at higher risk of developing AAD and patients taking a PPI were also at increased risk of AAD as was shown in the current study. The high incidence of AAD was attributed to the relatively prolonged follow-up period of 30 days and this may also be relevant to the current study, which documented the incidence of AAD for the duration of antibiotic therapy and up to 12 weeks after study recruitment.

Probiotic therapy may also reduce recurrent CDAD (268). A single site cohort controlled study of patients with CDAD treated with antibiotics alone or antibiotics in conjunction with LcS found that recurrent CDAD was significantly lower in the cohort taking probiotics (3.2% v 20.0%, $p=0.007$). This study is limited by its small sample size (66 patients in total) and its retrospective non-randomised design.

A large non-randomised study of 340 patients has compared the incidence of AAD after administration of *Lc*S to an entire hospital ward including the staff and an adjacent ward given routine clinical care (269). Only patients prescribed antibiotics on either ward were included in an analysis of AAD incidence. The incidence of AAD in the intervention group was 5% compared to 18.6% in the control group ($p<0.001$). Analysis of stool diversity showed a reduction in bacterial diversity and short chain fatty acid production in the routine care group.

The PLACIDE trial is the largest trial of probiotic use in AAD to date. It is a multicentre phase 3 trial of probiotic against placebo in patients aged 65 years or older (184). The study recruited 2974 patients from 17 420 patients screened. It differs from this current study as it recruited patients aged >65 years only. Patients were included if they had been prescribed antibiotics within 7 days prior to recruitment. There was no specific exclusion of recent antibiotic therapy. The probiotic supplement was continued for 21 days regardless of antibiotic therapy duration. Patients already taking probiotic supplements were included if they were willing to discontinue them at study entry. Patients with suspected acute pancreatitis or a history of mesenteric ischaemia were excluded from the PLACIDE study in light of the PROPATRIA trial (144), which found that probiotic

administration was associated with a worse outcome in those with acute severe pancreatitis. No patients with pancreatitis were included in our trial.

The PLACIDE study in patients > 65 years found no evidence of a reduction in AAD or CDAD with the addition of a freeze dried powder in a vegetarian capsule containing 6×10^{10} live bacteria: two strains of *Lactobacillus acidophilus* and two strains of bifidobacterium. AAD occurred in 12.9% of the probiotic group and 11.7% of the placebo group. In the current study no difference in the incidence of AAD was observed between patients greater and less than 65 years old (Tables 8 and 9). Although this study showed no reduction in AAD with a probiotic and it was a large randomized study, it had several limitations:

- study recruitment was permitted in subjects already using antibiotics for up to 7 days, and so these patients may have had alterations in gut microbiota prior to recruitment
- heterogeneity of antibiotic use with both intravenous and oral antibiotics being prescribed
- fixed duration of probiotic administration resulting in some subjects not taking probiotics for the duration of antibiotic therapy
- lower incidence of AAD and CDAD than expected resulting in the study being underpowered

There is evidence that patients with chronic kidney disease may have differences in gut flora compared with the general population (270). This results in differences in gut barrier function and may result in bacterial translocation (271) (272). These differences in gut flora may influence the effect of probiotics and make data from a general population difficult to extrapolate to renal patients. Out of all the studies described above, only the current study investigates the effect of probiotics on the prevention of AAD and CDAD in renal patients.

5.5 Conclusions

The role of probiotics in AAD is complex and is not fully understood. This double-blind randomized controlled study has failed to demonstrate any reduction in AAD or CDAD in patients with kidney disease who have commenced antibiotics within the previous 2 days. However, the study was underpowered which may result in small differences in AAD between the probiotic and placebo groups not being identified in this population. As recommended by the Cochrane Systematic Review, more research is required in this area, particularly in relation to probiotics and the prevention of AAD rather than CDAD alone.

Larger, multicentre studies are required in renal patients. Such studies should be at low risk to the study population as the current pilot study showed that there were no significant adverse events and in particular there were no episodes of lactobacillus bacteraemia during the study period, despite the high comorbidity and relative immunosuppression of the renal inpatient population.

5.6 Suggestions for further research

Further research is required into the immunomodulatory effects of probiotics with particular attention to genus and strain specific effects. Current research is difficult to compare due to differences in the probiotic intervention studied with yeasts, lactobacilli and preparations containing multiple strains included in meta-analyses.

The Human Microbiome Project has analysed the normal flora of the intestinal microbiota of 242 adults (273). It found that stool specimens displayed higher diversity than expected based on previous research although the gut microbiota seems to be a stable community in healthy subjects (274).. It has been hypothesised that changes in microbiota over time in the developed world has contributed to an increase in autoimmune disease such as asthma as part of the “hygiene hypothesis” although definitive research is lacking (275) (276).

Knowledge of the healthy microbiota may help our understanding of its role in disease states and therefore facilitate its manipulation for therapeutic purposes. Current research suggests decreased diversity of the gut microbiota in obesity although this may be accounted for by a high fat diet(277) (278). There is also emerging evidence that the

microbiota can be implicated in carcinogenesis in addition to more well established research showing a role in inflammatory bowel disease(279)(280). It is unclear whether these changes are causative in the pathogenesis of inflammatory bowel disease or secondary to underlying inflammation. The interaction of the microbiome and potential manipulation by probiotics or antibiotics requires further attention.

Chapter 6: Discussion and conclusions

The primary aim of this thesis was to further examine clinical problems in nephrology patients arising as a result of antibiotic use and infection.

6.1 Acute kidney injury before and after a change in antibiotic policy

The work detailed in Chapter 2 was a retrospective analysis of patients with acute kidney injury (AKI) requiring renal replacement therapy (RRT). We hypothesized that a change in antimicrobial guidance policies after an increase in *Clostridium difficile* associated disease (CDAD) cases and subsequent increase in gentamicin prescribing, could result in a rise in cases of gentamicin associated AKI who required renal replacement therapy (RRT).

The changes to antimicrobial guidelines were instituted in June 2008. Administered gentamicin doubled between August 2008 and August 2009 from 20 to 40 defined daily doses per 1000 bed days. The time periods analysed (Period 1 was 01/08/2007-31/01/2008 and Period 2 was 01/08/2008-31/01/2009) were chosen in order to minimize seasonal bias and to allow a "run in" period for the new guidelines.

Patients were identified retrospectively using the prospectively maintained intensive care unit and renal unit electronic patient records thus giving a full dataset of all patients receiving RRT for AKI within NHS Greater Glasgow and Clyde. There were no significant differences between the patient populations. In particular, the same proportion (43% patients) were prescribed gentamicin at any time during their admission to hospital and in fact, shorter courses of gentamicin were utilized during Period 2, perhaps reflecting increased antimicrobial stewardship and specific advice from the new antimicrobial guidelines to limit gentamicin prescribing to short courses only. There was no significant increase in gentamicin associated AKI requiring RRT between Period 1 and Period 2.

The data were further analysed as a whole in order to identify risk factors for gentamicin associated AKI. Patients were more likely to have undergone RRT in an ITU, have undergone surgery and had a more prolonged admission. Despite these adverse prognostic markers, there was no difference in mortality in those with gentamicin associated AKI and the remainder of the cohort.

Factors associated with deterioration in renal function from baseline were determined by binomial univariate then multivariate analysis of the entire cohort. Pre-existing renal impairment was associated with residual deterioration from baseline renal function following the episode of AKI. Patients who received RRT in a renal unit only were more likely to have deterioration from baseline renal function at discharge from hospital. This may reflect a difference in characteristics in these patients with an increased prevalence of patients with a primary renal disease such as glomerulonephritis. Gentamicin associated AKI was not associated with adverse renal outcomes.

Finally, the entire cohort was studied by univariate then multivariate regression analysis to establish risk factors for increased inpatient mortality. ITU admission and increasing age were independently associated with increased mortality whereas prior angiotensin converting enzyme inhibitor or angiotensin 2 receptor blocker use were associated with decreased risk of death on multivariate analysis. There was no association between gentamicin associated AKI and mortality although gentamicin use at any time was associated with an increased risk of death on univariate analysis. Patients with diabetes were associated with decreased risk of death on univariate analysis. Diabetic patients in this cohort were part of a highly selected subgroup judged as suitable for admission to either a renal unit or critical care unit and therefore are unlikely to be representative of the diabetic population as a whole.

These data support existing observational research that patients with pre-existing renal impairment are less likely to fully recover renal function after an episode of AKI and that requiring RRT in an ITU setting is associated with an increased risk of mortality. It is unique in its analysis of the effect of increased gentamicin prescribing in the hospital environment on a small group of critically ill patients.

One limitation of this study is that it was retrospective using an earlier time period as a control group in the initial analysis. Prospective research in this area would be very difficult due the unknown effects of omitting gentamicin or replacing it for an alternative antimicrobial. The requirement for gentamicin monitoring would result in blinding being impossible.

Comparison with other research is difficult due to varying definitions of AKI and the lack of a consensus on what constitutes gentamicin associated AKI. Gentamicin associated AKI in this study was defined as commencing gentamicin between 1 and 10 days prior to

requiring RRT and this population is unique as the main inclusion criterion is the requirement for RRT. As a result, only patients with the most severe AKI were included. Existing research of patients receiving gentamicin suggests that less than 5% patients require RRT for AKI in this context with 25% patients developing AKI of any severity. This study did not include patients with less severe gentamicin associated AKI which resolved without requiring RRT or those who developed AKI but were unsuitable for renal unit or critical care unit admission. Therefore, further research on the development of all forms of AKI in association with gentamicin use is required in order to assess the total risk of AKI associated with an increased use of gentamicin in the empiric treatment of sepsis in this era of increasing antibiotic resistance.

6.2 Staphylococcal bacteraemia in the renal unit.

Sepsis is second only to cardiovascular disease as the leading cause of death in HD patients and staphylococcal bacteraemia is the most common cause of bacteraemia in the renal inpatient unit and outpatient HD unit. Chapter 3 retrospectively compares the outcomes of patients with staphylococcal bacteraemia (methicillin sensitive *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococcal bacteraemia) in a renal inpatient population during 2010 and 2011 and compares the efficacy of different antibiotic regimens in the treatment of MSSA bacteraemia. The epidemiology of the *Staphylococcus aureus* bacteraemias in the patients in this study were examined using *spa* gene typing.

Bacteraemia in HD patients occurred at a similar rate to previous datasets. The majority (62.6%) of staphylococcal bacteraemias occurred in patients dialysing via tunnelled central venous catheters (TCVCs). TCVC use was associated with a higher risk of developing all forms of staphylococcal bacteraemia (MSSA, MRSA and coagulase negative staphylococcus bacteraemia). Sixty-five of the 248 (26.2%) patients from the cohort died within 6 months of a first episode of staphylococcal bacteraemia. Renal transplant recipients were less likely to die in the 6 months after a first episode of staphylococcal bacteraemia compared to patients requiring regular HD reflecting their better “prior to bacteraemia” life expectancy than patients requiring other forms of RRT.

Patients with MSSA bacteraemia had more frequent and more prolonged hospital stays in the 6 months following bacteraemia compared to MRSA bacteraemia although there were

no differences in the number of vascular access interventions, metastatic infection or mortality. This is in contrast to previous literature which reported increased mortality and higher healthcare costs associated with MRSA bacteraemia compared to MSSA. Although MRSA bacteraemia incidence is falling, MSSA bacteraemia incidence has remained relatively static and forms part of a current HEAT target for the reduction of healthcare associated infection (HCAI).

No difference in outcomes for MSSA bacteraemia treated with flucloxacillin or vancomycin (monotherapy or in conjunction with another antibiotic) was observed in this study. Vancomycin is often selected for its convenience in dosing in HD patients, as it can be administered intermittently on HD, thus avoiding a prolonged admission to hospital.

This study had several limitations. Firstly, retrospective interpretation of the analyses of coagulase negative staphylococcal bacteraemia was difficult due to bacteraemia being defined only on the basis of positive blood culture with no requirement for a systemic inflammatory response. Secondly the duration of antimicrobial therapy was confounded as patients who developed evidence of metastatic infection would be much more likely to be prescribed prolonged courses of antibiotic. Thirdly the small sample sizes of subpopulations in this analysis may have led to false negative results. Finally, although this study was limited by being retrospective, there are no prospective data comparing the efficacy of flucloxacillin and vancomycin in vivo on MSSA bacteraemia. Treatment selection and patient outcomes in different forms of staphylococcal bacteraemia should be an important focus for future research.

Spa gene typing showed that patients with recurrent SAB mostly become re-infected with the same genetic strain of *S. aureus*. This supports existing data suggesting that individual patients tend to be colonised with the same strain of *S. aureus*.

These data add to existing research confirming the increased risk of bacteraemia associated with TCVC use and support data suggesting consistent colonisation with the same strain of *S. aureus* may repeatedly causing disease in some patients. The need to achieve a reduction in the incidence of MSSA bacteraemia in renal units should become an increasing focus of care in the next few years. The first objective should be to achieve a higher rate of AVF use in incident and prevalent HD patients and reduce reliance on TCVCs. The second objective should be to develop more effective bundles of care in the prevention of MSSA bacteraemia in renal units and especially in HD patients with TCVCs. Future research

should consider the optimum combination of infection prevention strategies in this vulnerable patient group.

6.3 *Staphylococcus aureus* toxin gene positivity in colonisation and disease

Toxin gene positivity may be one of the factors accounting for increased virulence and disease severity of some strains of *S. aureus*. Disease secondary to *S. aureus* toxin genes can result in severe skin infection, necrotising pneumonia and desquamation of large areas of skin.

The data presented in Chapter 4 further examines the role of toxin gene positivity in two populations colonised by *S. aureus* (HD patients and healthy controls) and two populations with *S. aureus* disease (general practice patients with skin infections and patients with *S. aureus* bacteraemia). The prevalence of toxin gene positive *S. aureus* colonisation in the community is unknown, as is the proportion of disease caused by toxin gene positive *S. aureus*. The *spa* gene allows an epidemiological analysis of *S. aureus* to be performed. Different toxin genes are associated with specific *spa* types.

HD patients and healthy controls were prospectively screened for *S. aureus* by swabbing of the anterior nares into charcoal medium. *S. aureus* was confirmed using SAID agar plates forming distinctive green colonies then confirmed using latex agglutination.

Methicillin and mupirocin sensitivity was assessed in all samples using standard horse blood agar and antibiotic discs. Polymerase chain reaction followed by gel electrophoresis was performed to confirm findings. Toxin gene analysis was performed on all samples followed by *spa* gene analysis.

Overall, 30.3% of HD patients and 27.2% of healthy controls were colonised with *S. aureus* in the anterior nares. 9.1% of HD patients who were positive for *S. aureus* colonisation were colonised with MRSA and 2.9% of healthy controls positive for *S. aureus* colonisation were colonised with MRSA. 13.8% of the general practice skin samples were MRSA positive and 21.9% of the *S. aureus* bacteraemias were MRSA positive. There was evidence of recent medical intervention in many of the general

practice samples based on the source of infection given on the request form as either drain or surgical wound sites.

Toxin gene positivity was present in 15.0% of all *S. aureus* positive samples. There was a single PVL positive sample from a MSSA bacteraemia. There was a trend towards increased toxin gene positivity in patients with *S. aureus* infection and decreased toxin gene positivity in the healthy controls although there were no statistically significant results due to the small numbers involved. There was no significant difference in deprivation index between the four groups studied or between those colonised with *S. aureus* compared to those not colonised with *S. aureus*.

Spa gene analysis was compared in colonised and infected patients. There was no significant difference in genetic diversity between the groups. Genetic diversity in this population was similar to that reported in the literature.

The prevalence of *S. aureus* colonisation in these data is consistent with existing research data and, although it did not show a higher prevalence of S. *aureus* colonisation in HD patients, it was not powered for this purpose. It adds to current evidence that toxin gene positivity varies in different populations and larger prospective trials are required to give a true estimate of toxin gene positivity in *S. aureus* colonisation. Data from elsewhere in the UK suggests that colonisation with PVL positive MRSA in the community is rising although there was no evidence that this had occurred in this dataset. There was no convincing evidence of toxin gene positivity being associated with disease compared to colonised patients.

This study contributes to existing data suggesting that current understanding of the role of colonisation in invasive disease is far from complete. Its small size limits interpretation of results. Toxin gene positivity was low in this population and, although toxin genes are associated with disease, their role in causing infection requires further study.

6.4 Prevention of antibiotic associated diarrhoea using probiotic milk drink

The double blind randomised controlled trial presented in Chapter 5 examines the effect of a commercially available probiotic milk drink on the incidence of antibiotic associated diarrhoea (AAD) and *Clostridium difficile* associated disease (CDAD) in renal unit inpatients prescribed antibiotics. Patients were randomised to twice daily probiotic milk drink or placebo for the duration of antibiotic therapy continued for one week after completion of antibiotics. Forty- four patients were randomised to probiotic and 41 to placebo. There were no significant differences in baseline patient characteristics at randomisation.

This study did not show any evidence of a reduction in either AAD or CDAD in the probiotic study population. The incidence of AAD in the probiotic group was 36.36% compared to 34.14% in the placebo group. There were differences in which antibiotics were prescribed to the probiotic and placebo groups, but these difference were low risk antibiotics for AAD and CDAD and not higher-risk fluroquinolones, co-amoxiclav or cephalosporins. Patients prescribed proton pump inhibitor therapy were at higher risk of developing AAD on multivariate analysis in keeping with other studies.

Interpretation of the results of this randomised controlled trial was confounded by under-recruitment which resulted in the study being underpowered. Furthermore the power calculations for this study were performed using baseline CDAD rates which were higher than the CDAD rates observed in both the probiotic and control groups during the study period and in the general population.. This fall in CDAD rates in the renal and general patient population occurred as a result of changes in antibiotic prescribing during the study period and the introduction a bundle of preventive care measures for patients who developed loose stool of unknown cause.

Research into the effects of probiotic on AAD has increased dramatically since the study described in Chapter 5 began recruitment and there have been eight double blind randomised controlled trials published in the past 5 years. Data on the efficacy of probiotics in prevention of AAD and CDAD remains equivocal and is summarised in

Tables 6-1 and 6-2 below with the data from Chapter 5 included to allow comparison. From Table 6-1, it is clear that the studies are heterogenous and most of the studies were small. The majority of studies involved hospital inpatients with some studies limited to the elderly as they are known to be at increased risk of AAD. Agents used as the probiotic intervention were also variable with some studies using the yeast, *S. boulardii* and some using multiple preparations. Duration of probiotic therapy was variable, ranging from the duration of antibiotic therapy to two weeks after the antibiotic course was complete. Some studies continued probiotic therapy for a specified period of time regardless of antibiotic course duration. All studies were double blind with the exception of Hickson *et al* 2007, which was single blind. A placebo yogurt was administered by nursing staff in a medicine administration cup which was identical to that used for the intervention.

Table 6-2 examines outcomes in the study populations. The incidence of AAD in the control groups varied widely from 6.0% to 35.5%. Of the 17 studies, 7 had an incidence of AAD of less than 15% in the control group and 7 had an incidence of AAD of greater than 25%. There appears to be a trend towards higher AAD incidence in studies with a more prolonged follow-up period. This may partly explain the relatively high incidence of AAD in the study described in Chapter 5.

There were also highly variable background CDAD rates in the study populations with the incidence of CDAD in control groups ranging from 0 to 23.8%. This is difficult to interpret due to varying definitions of *C. difficile* positivity in the different studiess as some studies did not differentiate between positive *C. difficile* culture from solid stool in an asymptomatic patient and symptomatic CDAD. There was no definite trend of falling CDAD rates in these more recently reported studies.

There was no evidence of any trend in reducing the incidence of AAD or CDAD in the study described in Chapter 5. However the study was unique in studying renal inpatients which is a subgroup of hospitalised patients known to be at high risk of AAD and CDAD. The study was limited by its small sample size. The incidence of AAD, although high, is in keeping with some of the other studies published recently (Table 6-2). Further research into the role of probiotics in the prevention of either AAD or CDAD should focus on high risk patient populations and will need to be large multicentre studies if the studies are to provide an adequately powered study now that background CDAD rates are much lower than previously.

Table 6-1 Randomised controlled studies of probiotic against placebo in prevention of AAD: Patient characteristics, recruitment numbers, agent used and duration of intervention (181, 184) (281, 282), (283) (284) (285, 286) (287) (288, 289) (290) (291) (292)

Study Year	No. of patients		Clinical setting	Agent	Duration of intervention
	Study	Control			
Surawicz 1989	120	64	Hospital inpatients	S boulardii,	Within 48h of starting antibiotics continued for 2 weeks after course complete
McFarland 1995	97	96	Hospital inpatients taking beta lactam antibiotics	S boulardii	Within 72h of starting antibiotics continued for 3 days after stopping
Lewis 1998	33	36	Hospital inpatients aged >65	S. boulardii	Duration of antibiotics
Thomas 2001	133	134	Hospital inpatients	Lactobacillus GG	Within 24h of antibiotics then for 14 days
Plummer 2004	69	69	Hospital inpatients aged >65	lactobacillus and bifidobacterium	Within 72h of starting antibiotics, continued for 20 days
Can 2006	78	73	Hospitalised patients with no chronic illnesses	S. boulardii	Within 48h of antibiotic then during course
Beausoleil 2007	44	45	Hospital inpatients	L acidophilus, L casei	Within 48h of antibiotic then during course
Hickson 2007	69	66	Hospital inpatients	L. casei, L. bulgaricus, S. thermophilus	Within 48h of antibiotic continued for 1 week after course complete
Bravo 2008	41	45	Outpatients	S. boulardii	12 days
Safdar 2008	23	17	Hospitalised military veterans	L. acidophilus	14 days after stopping antibiotic
Wenus 2008	46	41	Hospital inpatients	L. rhamnosus GG, L. acidophilus, Bifidobacterium	Within 72h of starting antibiotics for 14 days
Gao 2010	171	84	Hospital inpatients of Asian ethnicity	L. acidophilus, L. casei	Within 36h of starting antibiotics continued for 5 days after antibiotic completed
Lonnermark 2010	80	83	Mix of hospital inpatients and outpatients	L. plantarum	Within 48h of starting antibiotics continued until a week after course complete
Psaradellis 2010	216	221	Hospital inpatients	L. acidophilus, L. casei	Within 24h of starting antibiotics continued for 5 days after course complete
Pozzoni 2012	141	134	Hospital inpatients aged >50	S. boulardii	Within 48h of starting antibiotics continued for 7 days after course complete
Allen 2013	1493	1488	Hospital inpatients aged >65	L. acidophilus, Bifidobacterium	Within 7 days of starting antibiotic continued for 21 days
Helps 2013	44	41	Renal unit inpatients	L. casei	Within 48h of starting antibiotics continued for 7 days after course complete

Table 6-2 Randomised controlled studies of probiotic against placebo in prevention of AAD: incidence of AAD and CDAD with duration of follow-up (181, 184) (182, 281, 282) (283) (284) (285, 286) (287) (288, 289) (290) (291) (292)

Study Year	AAD		CDAD		Follow up
	Probiotic	Control	Probiotic	Control	
Surawicz 1989	11 (9.2%)	14 (21.9%)	5 (4.2%)	5 (7.8%)	Up to 25 days after stopping antibiotics
McFarland 1995	7 (7.2%)	14 (14.6%)	Not assessed		7 weeks after stopping antibiotic
Lewis 1998	7 (21.2%)	5 (13.9%)	5 (15.2%)	3 (8.3%)	Antibiotic duration
Thomas 2001	39 (29.3%)	40 (29.9%)	not assessed		7 days after last study dose
Plummer 2004	15 (23.1%)	15 (23.1%)	2 (2.9%)	5 (7.3%)	Not specified
Can 2006	1 (1.4%)	7 (9%)	0	2 (3%)	4 weeks after stopping antibiotics
Beausoleil 2007	7 (15.9%)	16 (35.5%)	1 (2.3%)	7 (15.6%)	21 days after last antibiotic dose
Hickson 2007	7 (10.1%)	19 (28.8%)	0	9 (13.6%)	5 weeks after stopping antibiotic
Bravo 2008	4 (9.8%)	5 (11.1%)	not assessed		9 days after last study drug dose
Safdar 2008	4 (17.%)	6 (35.3%)	0	1 (5.6%)	Not specified
Wenus 2008	2 (5.9%)	8 (27.6%)	0	1 (2.4%)	Not specified
Gao 2010	37 (44.0%)	37 (21.6%)	9 (5.3%)	20 (23.8%)	26 days after stopping antibiotics
Lonnermark 2010	6 (7.5%)	5 (6.0%)	1 (1.3%)	0	7-10 days after stopping antibiotics
Psaradellis 2010	47 (21.8%)	70 (31.7%)	1 (6.2%)	4 (13.3%)	26 days after stopping antibiotics
Pozzoni 2012	16 (11.3%)	13 (9.7%)	3 (2.1%)	2 (1.5%)	4 weeks after stopping antibiotics
Allen 2013	159 (10.8%)	153 (10.4%)	12 (0.8%)	17 (1.2%)	8 weeks after recruitment
Helps 2013	16 (36.36%)	14 (34.1%)	0	2 (5.0%)	12 weeks after recruitment

6.5 Conclusion

Sepsis remains second to cardiovascular disease in the reported causes of death in the RRT population. The prevention of infection and avoidance of harm secondary to the treatment of infection are particularly relevant to the renal unit population due to the high incidence of infective complications in this patient group.

This thesis has shown that the wider use of empiric gentamicin in hospitalized patients did not result in an increased incidence of dialysis dependent antibiotic associated AKI, which is reassuring. In contrast, the persistent, high rates of different forms of Staphylococcal bacteraemia in renal inpatients is disappointing given the efforts which have been made in attempting to increase the proportion of patients using an AVF for HD and the provision of bundles of care aimed at prevention of infection, especially in patients using a TCVC for vascular access for HD.

The study of the role of toxin gene positivity in patients colonised by *S. aureus* (HD patients and healthy controls) and patients with *S. aureus* disease (general practice patients with skin infections and patients with *S. aureus* bacteraemia) found that the frequency of toxin gene positivity did not differ significantly among the different patient populations. The role of the gut microbiome in the prevention and causation of disease is poorly understood but the use of probiotics in optimizing the gut flora seems intuitive. A small randomized double blind trial has failed to show any trend for a reduction in AAD or CDAD in a renal inpatient population commencing antibiotics within 2 days of starting a probiotic. When this study is added to other randomized controlled trials definitive evidence of benefit of probiotic therapy in the prevention of AAD and CDAD or in its treatment remains unproven.

This thesis adds to the growing body of work towards safer prescribing and reduction in risk associated with treatment of infection. Questions remain regarding the optimum approaches in preventing *S. aureus* bacteraemia and AAD and CDAD in the renal patient population. Future research is likely to focus on the ideal bundle of measures for maximum risk reduction in addition to novel approaches such as vaccination and decolonization strategies.

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Appendices

8.1 Patient characteristics and toxin gene positivity of *S. aureus* isolates originating from the community.

Patient number	Age	swab location	sex	MRSA	PVL	ETA	ETB	TST
1	57	shoulder	F	0	0	1	1	0
2	59	ankle	M	0	0	1	0	0
3	43	abdominal wound	F	0	0	1	0	0
4	81	hip	F	0	0	0	0	1
5	69	skin	F	0	0	0	0	1
6	69	stump site	F	0	0	0	0	1
7	51	abdominal wound	F	0	0	0	0	1
8	29	toe	F	0	0	0	0	1
9	28	ear	M	0	0	0	0	0
10	22	pilonidal sinus	M	0	0	0	0	0
11	78	wound	F	0	0	0	0	0
12	80	leg ulcer	F	0	0	0	0	0
13	39	ear	F	0	0	0	0	0
14	72	PEG* tube	F	1	0	0	0	0
15	56	leg ulcer	M	0	0	0	0	0
16	71	ear	M	0	0	0	0	0
17	92	great toe	F	0	0	0	0	0
18	89	calf wound	F	0	0	0	0	0
19	75	foot	M	0	0	0	0	0
20	40	finger	F	0	0	0	0	0
21	54	abdominal wound	M	0	0	0	0	0
22	95	NK	F	0	0	0	0	0
23	63	ear	M	0	0	0	0	0
24	36	heel	M	0	0	0	0	0
25	32	pilonidal sinus	M	0	0	0	0	0
26	57	toe	M	0	0	0	0	0
27	53	ear	M	0	0	0	0	0
28	74	leg wound	F	0	0	0	0	0
29	62	wrist	F	0	0	0	0	0
30	80	wound	F	0	0	0	0	0
31	55	ear	M	0	0	0	0	0
32	39	heel	F	1	0	0	0	0
33	38	wound	F	0	0	0	0	0
34	74	wound	F	0	0	0	0	0
35	77	sacral wound	F	0	0	0	0	0
36	48	nail bed	F	0	0	0	0	0
37	63	leg ulcer	M	0	0	0	0	0
38	30	nk	F	0	0	0	0	0
39	79	leg	F	0	0	0	0	0
40	74	foot	F	0	0	0	0	0
41	28	head	M	0	0	0	0	0
42	45	wound	M	0	0	0	0	0
43	42	skin	F	0	0	0	0	0
44	64	ankle	F	1	0	0	0	0
45	74	leg wound	F	0	0	0	0	0
46	56	groin	F	0	0	0	0	0
47	83	wound	M	0	0	0	0	0

48	80	leg ulcer	M	0	0	0	0	0
49	72	nose	F	1	0	0	0	0
50	66	chest drain site	M	1	0	0	0	0
51	55	abdominal wound	F	1	0	0	0	0
52	30	toe	M	0	0	0	0	0
53	64	foot	M	1	0	0	0	0
54	82	heel pressure sore	F	0	0	0	0	0
55	22	ankle	F	0	0	0	0	0
56	46	leg	M	0	0	0	0	0
57	66	leg wound	F	0	0	0	0	0
58	38	spine wound	F	1	0	0	0	0

* Percutaneous endoscopic gastrostomy

8.2 Clinical Research Form: Probiotics study Chapter 6

Patient Information Sticker		Randomisation number: Date: Location: Telephone number: Alternative contact:

Inclusion criteria:

1	Aged over 18	
2	Inpatient in renal unit	
3	To start or has started a course of antibiotic other than metronidazole or vancomycin in the past 48h	

Exclusion criteria:

1	Diarrhoea on admission or in the previous week (3 or more loose bowel motions in 24h)	
2	Lactose intolerance or intolerance to dairy products	
3	Regular probiotic use in the 4 weeks prior to admission	
4	Unable to give consent within 48h of starting antibiotics	
5	Antibiotic use in the past 4 weeks	
6	Induction dose immunosuppression	
7	Active inflammatory bowel disease or bowel surgery less than 6 months ago	

Consent form completed x3 (copy overleaf) **Y/N**

Baseline patient characteristics:

Underlying renal disease: _____

RRT: Haemodialysis/PD/transplant _____ duration
HD access: TCVC/Temp line/Graft/AVF
Duration on current mode of RRT

Co-morbidity:
Hypertension Y/N
IHD Y/N
Cerebrovascular disease Y/N
Diabetes mellitus Y/N
Type 1 or type 2? _____
Insulin Y/N
Valvular heart disease Y/N
Asthma/COPD Y/N
Vasculitis Y/N
Details_____
Malignancy Y/N
Details_____
Previous C. difficile diarrhoea Y/N
When_____
Other _____

Medications:
Immunosuppression Y/N
Details_____
PPI Y/N
Preparation/dose _____
Laxatives Y/N
Anti diarrhoeal (loperamide, codeine etc) Y/N

Current admission

Initial stool sample sent for reference? Y/N Date:_____

Site of infection:

Antibiotic	Dose	Start date	Stop date	Reason for change

Microbiology results:

Date taken	Site of culture	Date of result	Organism	Sensitivities

Diarrhoea: defined as 3 or more loose bowel motions in 24 hours

Present during antibiotics? Y/N

Date: _____

Culture results:

Date	Result	Action taken

Present after finishing antibiotics?

Date: Y/N

Culture results:

Date	Result	Action taken

Follow up:

1 week after stopping antibiotics

Compliance with milk drink Y/N Presence of diarrhoea Y/N

Culture sent if required Y/N Culture result _____

2 weeks after stopping antibiotics

Presence of diarrhoea Y/N Start date:_____ End date: _____

Culture sent if required Y/N Culture result _____

8.3 Patient Information Sheet: Probiotics Study Chapter 6

Study Title: Prospective randomised double blind study of efficacy of probiotic milk drink in reducing the incidence of antibiotic associated diarrrhoea and Clostridium difficile

(Does probiotic milk drink reduce antibiotic associated diarrhoea?)

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. (Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study).
Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
It is known that Antibiotic Associated Diarrhoea (AAD) and in particular Clostridium Difficile (C. difficile) is common in hospital patients, especially those started on broad-spectrum antibiotics, such as co-amoxiclav (augmentin) or ciprofloxacin. As many as a quarter of those prescribed these antibiotics may experience AAD or C. difficile.

There is some evidence from scientific studies which suggests that probiotic drinks might help reduce the incidence of antibiotic associated infections and C. difficile infections although these are not conclusive.

Why have I been invited?
We are asking all patients who are admitted to the Renal Wards and are prescribed certain antibiotics and are over 18 to participate in a study of whether probiotic drinks will reduce the incidence of antibiotic associated diarrhoea and c difficile.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
If you agree to take part you will be asked to take either Yakult (a probiotic milk drink) or sterile milk drink twice daily. There should be no way to tell which type of drink you will be drinking and the doctors and nurses will not know either. This will start within 48h of starting the antibiotic and continue for 7 days after stopping the antibiotic.
We will also obtain some basic medical information from your hospital records. One of the doctors will also ask you some additional questions and to make a decision whether you are suitable for the study.

What will I have to do?
Other than take the drink two times per day, there will be very little else for you to do. If you develop diarrhoea while in hospital, there will be samples sent to the laboratory looking for C. difficile. We will take a stool sample from you at the beginning of the study so that it can be analysed later if you develop diarrhoea. If you develop diarrhoea while at

home, we will ask you to let us know, so that samples can be sent for analysis. We will ask you to keep taking the drink for 7 days after stopping antibiotics.

What are the alternatives?
If you choose, you can stop taking the drink at any time. This will not affect your treatment while in hospital or afterwards.

What are the possible disadvantages and risks of taking part?
You may find that you do not like the milkshake or are intolerant of the lactose it contains. There have been some case reports of people possibly developing infections as a result of probiotic yogurts. Those on high doses of medications to suppress the immune system are not being included in this study.

What are the side-effects of any treatment received while taking part?
Other than those mentioned above, there are no additional side-effects.

What are the possible benefits of taking part?
It is hoped that the incidence of antibiotic associated diarrhoea will be reduced by the use of probiotic yogurt although you may not benefit personally from this study. We hope to use the information gathered to find out if probiotic drinks reduce the incidence of antibiotic associated diarrhoea and clostridium difficile in patients in the Renal Unit. If you do develop AAD or C. difficile, you will, of course, be treated in the conventional way.

What happens when the research study stops?
When the study stops, we hope to have developed a policy for the use of probiotic yogurts in the renal unit. You will have been contacted 1 month and 6 months after starting the study to check there have not been any problems in the meantime.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

This completes Part 1. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What if relevant new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you and discuss whether you should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study he may ask you to sign an updated consent form. If this happens, your research doctor might consider you should withdraw from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, we will tell you and arrange your continuing care.

What will happen if I don't want to carry on with the study?

You can withdraw from treatment at any time. This will not affect the care you receive. Information collected may still be used. Any stored blood or tissue samples that can still be identified as yours will be destroyed if you wish.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact number supplied). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised. Your GP will be notified that you are taking part in this study as part of your hospital discharge letter.

What will happen to any samples I give?

An initial sample of your bowel motions will be taken and stored in the microbiology department so that it can be analysed if you develop diarrhoea to confirm that any bacteria found are new. If you develop diarrhoea, samples of your diarrhoea will be analysed by the microbiology laboratory and disposed of in the usual way.

What will happen to the results of the research study?

We plan to publish the results of this study in a medical journal.

Who is organising and funding the research?

Renal Unit doctors of the Glasgow Royal Infirmary designed this study. There is no external funding for the study and none of your doctors will be paid for including you in the study.

The study design has been assessed and passed by the independent Local Research Ethics Committee of Glasgow Royal Infirmary.

Further information and contact details:

Dr Aileen Helps, Research Fellow, or Dr Robert Mactier, Consultant Nephrologist
Both contactable at 0141-211 0566

Study Title: Prospective randomised double blind study of efficacy of probiotic milk drink (Yakult) in reducing the incidence of antibiotic associated diarrhoea and Clostridium difficile

(Does probiotic milk drink reduce antibiotic associated diarrhoea?)

Name of Researchers: Dr R.A. Mactier, Dr A Helps

Please initial box

1. I confirm that I have read and understand the information sheet dated 25th February 2009 (version 4) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes