The Ecology of Antibiotic Use in the ICU: Homogeneous Prescribing of Cefepime but Not Tazocin Selects for Antibiotic Resistant Infection

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Abstract

**Background:** Antibiotic homogeneity is thought to drive resistance but in vivo data are lacking. In this study, we determined the impact of antibiotic homogeneity per se, and of cefepime versus antipseudomonal penicillin/β-lactamase inhibitor combinations (APP-β), on the likelihood of infection or colonisation with antibiotic resistant bacteria and/or two commonly resistant nosocomial pathogens (mecillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa). A secondary question was whether antibiotic cycling was associated with adverse outcomes including mortality, length of stay, and antibiotic resistance.

**Methods:** We evaluated clinical and microbiological outcomes in two similar metropolitan ICUs, which both alternated cefepime with APP-β in four-month cycles. All microbiological isolates and commensal samples were analysed for the presence of antibiotic-resistant bacteria including MRSA and *P. aeruginosa*.

**Results:** Length of stay, mortality and overall antibiotic resistance were unchanged after sixteen months. However, increased colonisation and infection by antibiotic-resistant bacteria were observed in cefepime cycles, returning to baseline in APP-β cycles. Cefepime was the strongest risk factor for acquisition of antibiotic-resistant infection.

**Conclusions:** Ecological effects of different β-lactam antibiotics may be more important than specific activity against the causative agents or the effect of antibiotic homogeneity in selection for antibiotic resistance. This has important implications for antibiotic policy.

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Introduction

Immediate effective antibiotic therapy is one of the most important intervention in severe sepsis, but ultimate survival is determined before cultures become positive and antibiotic susceptibilities available [1,2]. Broad-spectrum antibiotics such as carbapenems (e.g. meropenem), antipseudomonal penicillin/β-lactamase inhibitor combinations (APP-β) like piperacillin/tazobactam (TZP) and ticarcillin/clavulanate (TIM), and broad-spectrum cephalosporins such as cefepime are commonly used, but are restricted in many countries, with the aim of minimising development of antibiotic resistance.

The clinical benefit of antibiotic-intensive strategies such as selective decontamination of the digestive tract [3] comes at the cost of increased antibiotic resistance in the microflora [4]. Conversely, antibiotic restriction may reduce antibiotic resistance. In the “antibiotic cycling” strategy, potent antibiotics are alternated in order to allow specific resistance to subside. Initially promising reports [5] have been followed by numerous studies with mixed results. A study comparing 4-month cycles of carbapenems, third-generation cephalosporins and APP-β sug-
suggested a relationship between highly homogeneous antibiotic selection pressure and resistant infection [6], while another using carbapenems, quinolones, cephalosporins and APP-β found no increase in resistance in the microflora during or after the cycling period [7]. Expert reviewers conclude that more data are needed, as many questions remain unanswered [8,9], but mathematical models of antibiotic cycling predict that homogeneous selection pressure will increase antibiotic resistance [10,11].

Our primary aim was to determine whether cycles of relatively homogeneous β-lactam use, alternating cefepime and APP-β as the core of empiric antibiotic therapy for sepsis in ICU, resulted in increased antibiotic resistance. We defined this as the acquisition at any site of either MRSA or P. aeruginosa (two commonly resistant nosocomial pathogens) or of any bacteria resistant to cefepime, APP-β, or gentamicin, and considered these separately. A secondary aim was to determine whether the institution of antibiotic cycling in ICU was associated with adverse clinical outcomes, specifically relating to mortality and length of stay, in light of previous findings of increase mortality associated with empiric use of cefepime in the critically ill [12].

**Methods**

**Study Design and Population**

We varied the β-lactam component (cefepime vs APP-β) of empiric antibiotic prescribing in four month cycles in two well-matched high-acuity metropolitan Australian ICUs. No attempt was made to influence prescribing for known or suspected aetiologies, but those judged in need of empiric broad-spectrum β-lactam therapy by the caring physician received cycle-specified β-lactam (cefepime or APP-β) with other antibiotics as deemed appropriate. Standard infection control procedures applied, according to matching guidelines in both institutions. Approval to conduct the study, under a waiver of consent, was obtained from the relevant Human Research Ethics Committees of the Sydney West Area Health Service and the Royal Brisbane and Women’s Hospital.

There were four consecutive cycles in Unit 1 (Westmead, WM), beginning in April 2004 (cefepime), August (APP-β), December (cefepime) and April 2005 (APP-β), respectively (Figure 1), and three in Unit 2 (Royal Brisbane and Women’s Hospital, RBWH), beginning in August 2004 (APP-β), December 2004 (cefepime) and April 2005 (APP-β). For a short period (February to March 2005), nationwide shortages of piperacillin/tazobactam (TZP) meant substitution with ticarcillin/clavulanate (TIM) as the APP-β, as these agents are generally regarded as closely comparable in their antibacterial spectra [13]. Hereafter the term APP-β is used for both.

Of the 1987 admissions to both ICUs in this period, 1194 were for at least 48 hours. Of these, 236 were admitted within 48 h of hospital arrival and received only cefepime or APP-β, or no antibiotics at all, by the end of their first week in ICU. In 206 of these (72%), late clinical followup and microbiological surveillance (see below) at all timepoints was complete. This group represents patients most clearly and directly subject to cycle-specific antibiotic influences. Data collected included age, sex, admission Acute Physiology and Chronic Health Evaluation (APACHE II) score, reason for admission, surgical and other interventions, ventilation and dialysis days, presence of central venous catheters, dialysis catheters, hospital and ICU length of stay (LOS), mortality, detailed antibiotic treatment, and microbiological information (Table 1). The standardised APACHE score remains the premier predictor of ICU and hospital mortality for ICU admissions with high predictive value [14] and high inter-rater reliability [15].

**Figure 1. β-lactam use in cycle periods.** Relative distribution of antibiotic use (defined daily doses) among the major β-lactams: cefepime (FEP), antipseudomonal penicillin combinations (APP-β), meropenem (MEM), and ceftriaxone or cefotaxime (CTX) use in the combined Units (n = 1987).

APACHE II [16] incorporates a range of acute (temperature, mean arterial pressure, blood pH, heart and respiratory rate, arterial-alveolar oxygen gradient, serum Na, K, Creatinine, white blood count, haematocrit, Glasgow Coma Score, presence of acute renal failure) and chronic indicators (age, hepatic failure, encephalopathy, cirrhosis, portal hypertension, heart failure, dialysis, chronic respiratory failure and respiratory conditions, and immunosuppression including leukaemia, lymphoma or AIDS). Antibiotic use, recorded as numbers of patients treated and as defined daily doses (DDD), included days on which the antibiotic regimen started and ended. Antibiotic doses were adjusted up and down from the standard guidelines by the duty intensive care specialist on the basis of usual pharmacokinetic considerations (renal and hepatic function, dialysis, volumes of distribution, protein binding) in individual patients, but antibiotic levels in blood and tissues were routinely measured only for aminoglycosides and vancomycin. All antibiotics, including vancomycin and gentamicin, were otherwise dosed and monitored according to standard Australian guidelines [13]. Cefepime dosing was recommended at 6 g/day (2 g every 8 h) for severe infection, and piperacillin 4 g plus tazobactam 500 mg (TZP 4.5 g) every 8 h or ticarcillin 3 g/clavulanate 100 mg (TIM 3.1 g) every 6 h for an average person with normal renal function.

**Microbiological Analysis**

Positive cultures from all clinically indicated specimens submitted to the diagnostic laboratory were identified. Respiratory tract isolates were reported as significant if a pathogen was identified as sufficiently numerous in semi-quantitative assays (≥10^5 cfu/mL in non-bronchoscopic or directed bronchoalveolar lavage) or was predominant in a purulent sputum specimen without significant epithelial contamination evident (<10 epithelial cells per high-power field) on microscopy. Isolates were also deemed significant if
Table 1. Patient characteristics.

| Characteristic | Unit 1 (n = 1135) | Unit 2 (n = 852) | p^ | All admissions (n = 1987) |
|---------------|------------------|-----------------|---|--------------------------|
| Age (years) median (IQR) | 59.5 (41.1–71.6) | 57.8 (40.1–70.9) | 0.203 | 58.7 (40.8–71.3) |
| Male, n (%) | 690 (60.8) | 522 (61.3) | 0.433 | 1212 (61.0) |
| ICU LOS (days) median (IQR) | 3.2 (1.5–7.3) | 2.2 (1.0–6.4) | <0.001 | 2.8 (1.2–6.9) |
| HOS LOS (days) median (IQR) | 175 (6.6–39.0) | 163 (7.1–32.7) | 0.529 | 170.7 (7.0–36.1) |
| APACHE II median (IQR) | 20.0 (14.0–26.0) | 18.0 (13.0–24.8) | 0.005 | 19.0 (13.0–25.0) |
| Admission from community <48 h, n (%) | 664 (58.5) | 613 (71.9) | <0.001 | 1277 (64.3) |
| Admission category: Trauma/surgical, n (%) | 650 (57.3) | 472 (55.4) | 0.411 | 1122 (56.5) |
| Medical, n (%) | 485* (42.7) | 380 (44.6) | 0.411 | 865 (43.5) |
| ICU Readmission, n (%) | 47 (4.1) | 45 (5.3) | 0.138 | 92 (4.6) |
| Multiple ICU Readmissions, n (%) | 19 (1.7) | 10 (1.2) | 0.234 | 29 (1.5) |
| Operative Intervention, n (%) | 314 (27.7) | 540* (63.4) | <0.001 | 854 (43.0) |
| Intracranial drain or monitor, n (%) | 56 (4.9) | 74 (8.7) | 0.001 | 130 (6.5) |
| Intercostal Drain, n (%) | 40 (3.5) | 80 (9.4) | <0.001 | 120 (6.0) |
| Nasogastic catheter, n (%) | 864 (76.1) | 657 (77.1) | 0.323 | 1521 (76.5) |
| Endotracheal Tube or Tracheostomy, n (%) | 842 (74.2) | 629 (73.8) | 0.448 | 1471 (74.0) |
| Urinary catheter, n (%) | 913 (80.4) | 819 (96.1) | <0.001 | 1732 (87.2) |
| Arterial catheter, n (%) | 889 (78.3) | 797 (93.5) | <0.001 | 1686 (84.9) |
| Central venous catheter, n (%) | 836 (73.7) | 594 (69.7) | 0.3 | 1430 (72.0) |
| Other vascular (dialytic) catheter, n (%) | 95 (8.4) | 86 (10.1) | 0.207 | 181 (9.1) |
| Dialysis, n (%) | 92 (8.1) | 84 (9.9) | 0.176 | 176 (8.9) |
| ICU Mortality, n (%) | 137 (12.1) | 112 (13.1) | 0.258 | 249 (12.5) |
| Hospital Mortality, n (%) | 165 (14.5) | 136 (16.0) | 0.208 | 301 (15.1) |

Patient characteristics of all admissions.

^Mann-Whitney U test or Chi-Squared analysis.
*Includes tracheostomies.
1Includes haematology/marrow transplant (~1% of admissions to both units);

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grown from normally sterile sites (e.g., bloodstream) or if predominant in the specimen on microscopy and culture (e.g., deep tissues) or if pyuria was present and >10^6 cfu/mL of a single organism was grown from urine. The reporting laboratory must also have issued full identification and susceptibility data on the basis of established standard laboratory criteria in both centres, in accordance with established definitions [17] by prior agreement between investigators in both units.

All specimens were given unique numbers and de-identified before processing. Surveillance samples (perineal swab and endotracheal aspirate) obtained on admission and twice weekly thereafter were submitted to the centralised reference laboratories serving each unit (Unit 1: Centre for Infectious Diseases and Microbiology (CIDM) Laboratory Services at Westmead Hospital, Sydney; Unit 2: Queensland Pathology at the Royal Brisbane and Women’s Hospital, Brisbane), and inoculated into nutrient broth overnight, resuspended in 20% glycerol in nutrient broth (GNB, Women’s Hospital, Brisbane), and inoculated into nutrient broth at 37 °C for transfer to CIDM. A loopful of each frozen sample was resuspended in nutrient broth at 37 °C for 90 min aerobically before inoculation onto solid media. *Pseudomonas aeruginosa* was confirmed by growth on Pseudomonas Isolation Agar (Becton Dickinson) [18] and oxidase activity (Oxoid), and methicillin-resistant *Staphylococcus aureus* (MRSA) by growth on Brilliance MRSA Agar (Oxoid) [19] with confirmatory testing using the BactStaph reagent (Remel) [20]. MRSA isolates were subtyped by Smal PFGE as previously described [21] and analysed for relatedness as per standard criteria [22]. *Enterobacteriaceae* were detected by their morphology on chromogenic agar (CHROMagar Orientation) [23], and tested for growth in the presence of ticarcillin/clavulanate (120/2 µg/mL), gentamicin (10 µg/mL) or cefepime (64 µg/mL) incorporated into the agar. Colonies with the typical appearance of *E. coli* or *Klebsiella/Enterobacter/Serratia/Citrobacter spp. on CHROMagar, in accordance with the Australian National Accreditation Testing Authority approved methodology in each laboratory and with the media manufacturer’s guidelines, were designated “*Enterobacteriaceae*” after testing to exclude oxidase activity. All clinical isolates of *P. aeruginosa* and all TIM-resistant *P. aeruginosa* isolates from surveillance samples, as well a random subset of TIM-resistant *Enterobacteriaceae*, were also tested against TZP. No significant differences were found in antimicrobial susceptibility so TIM was retained as the APP-β resistance marker. Unless otherwise specified in the text, ‘resistant’ or ‘resistance’ refers to APP-β, gentamicin and/or cefepime, in the above concentrations. All MRSA isolates were resistant to these three antibiotics, but *P. aeruginosa* isolates were specified as ‘resistant’ if resistant to any.

Statistical Analysis

Acquisition of infection and/or colonisation by antibiotic-resistant bacteria and by *P. aeruginosa* and methicillin-resistant *S. aureus* was the primary outcome for analysis. Each significant isolate and each antibiotic-resistant colonisation was counted only once per admission. Data were analysed separately for both hospitals before being pooled, as the differences between the two
Units in terms of casemix and basic demographics were minor (see Table 1). Statistical analysis was performed using SPSS (v18, SPSS Inc.). Proportions were compared using the Mann-Whitney U, one-way analysis of variance (ANOVA) or Chi-Squared tests, as specified. Analysis of multiple predictors for ICU clinical and bacteriological outcomes was performed using step-wise backwards logistic regression models, considering only those factors significant at p≤0.10 in univariate analysis, and retaining only factors significant at p≤0.05 in the final model.

**Results**

**The Study Population**

Admissions to Unit 1 (n = 1135) and Unit 2 (n = 852) were similar in casemix and other key characteristics (Table 1), and were combined for most analyses. Illness severity was moderately high, with median APACHE II scores of 20 (IQR 14–26) vs. 18 (IQR 13–25), ICU mortality rates of 12.1% vs. 13.1% and inhospital mortality of 14.5% vs. 16.0% for Units 1 and 2, respectively. Predictors of mortality within the set of all admissions (n = 1987) by multivariate analysis were age≥65 (OR 1.45; 95% CI 1.10–1.90), endotracheal intubation for mechanical ventilation (OR 1.59; 95% CI 1.14–2.23) and renal failure requiring dialysis (OR 2.92; 95% CI 1.89–4.53), all p<0.01. Surgical admissions were associated with a relatively lower mortality risk (OR 0.49; 95% CI 0.37–0.65; p<0.001).

**Antibiotic Homogeneity and Main Outcomes**

Antibiotic homogeneity was high: cycle-specified drug comprised >60% of major β-lactam antibiotic use in respective on-cycles and <15% in off-cycles (Figure 1). Other antibiotic use was generally consistent between cycles (Figure 2). Unit 2 had significantly more admissions from the community (71.9 vs 58.5%) and a higher incidence of urinary catheterisation (96.1 vs 80.4%), but there were no other important differences in major demographic or casemix variables, and no significant differences in infection rates, mortality or length of stay, either between cycles or from beginning to end-study, when data from both Units were considered together (Table 2 and 3). The final cycle had the most patients, the highest proportion mechanically ventilated (Table 2), the highest antibiotic use, and the highest treatment intensity (cycle 1:2449 DDD/1965 patient days, ratio 1.25; cycle 2:24656/3737, 1.25; cycle 3:3012/3656, 1.37; cycle 4:4030/3562, 1.44; Table 4). Despite this, the proportion of admissions complicated by infection in the last (APP-β) cycle was unchanged in Unit 1, and reduced in Unit 2 when compared to previous cycles (Table 3).

**Risk of Infection by Resistant Bacteria is Higher in Cefepime Cycles**

In order to determine the impact of the two different β-lactam regimens, we compared the risk of infection and/or colonisation with either MRSA or P. aeruginosa or other antibiotic resistant bacteria. Two hundred and twenty-three admissions in all were complicated at some stage by resistant infection. Of all infecting bacteria resistant to any of the three first-line antibiotics, APP-β (isolated from n = 121, 54.3% of admissions) and gentamicin resistance (132, 59.2%) were most common. Cefepime resistance was rare among Enterobacteriaceae (2, 0.9%) and P. aeruginosa (11, 4.9%). A cefepime-resistant infection complicated only 32 (14.3%) admissions. Forty-one (18.4%) of the 223 admissions complicated by resistant infection were complicated by antibiotic-resistant P. aeruginosa and 81/223 (36.3%) by MRSA.

Although there was no difference in the proportion of admissions complicated by any infection at all, the proportion of admissions complicated by antibiotic-resistant infection was more than twice as high in cefepime cycles as in APP-β cycles (164.1 vs 74.2 per 1000 admissions; p<0.001). MRSA infection complicated significantly more cefepime than APP-β cycle admissions (116.3 vs. 72.2/1000 admissions; p 0.01), and this was also evident between individual cycles (Table 3). P. aeruginosa infection (any/all, including gentamicin and APP-β susceptible) complicated a greater proportion of admissions in cefepime than APP-β cycles (Table 3), and this was statistically significant in Unit 1, although analysis according to resistance phenotype was not statistically meaningful. A similar trend in both Units for increased infection by (any/all) P. aeruginosa in cefepime cycles (cefepime vs APP-β: 98.3 vs 69.3/1000; Table 3) was not statistically significant (p 0.07).

Fourteen cefepime-cycle admissions were complicated by antibiotic-resistant bacteraemia compared to nine APP-β-cycle admissions although the difference between these small numbers was not significant (16.6 vs 7.9 per 1000 admissions; p 0.089). About a third of all bacteraemias (10/35, Unit 1; 5/12, Unit 2) were due to Enterobacteriaceae but few were antibiotic-resistant and these were not further analysed.

**Risk of Colonisation by Resistant Bacteria is higher in Cefepime Cycles**

Increased infection in those exposed to cefepime should be associated with increased colonisation rates. We identified admissions direct from the community who received either specified β-lactam (and no off-cycle β-lactam) for >48 hours before sampling in the first week of admission, or no antibiotics at all (60/152, 39% of APP-β cycle admissions; 58/134, 43% of cefepime cycle admissions). This subset (n = 286), with higher admission APACHE II scores and ICU length of stay (Table S1), most accurately reflects antibiotic influence. When data were pooled and compared by cycle type, there were no significant differences in overall proportions of admissions in which MRSA or P. aeruginosa was isolated from surveillance samples (Table 3). However, when the first, middle and final thirds of each cycle are plotted to account for ‘washout’ from the preceding cycle, a trend...
Table 2. Patient characteristics in each cycle.

| Characteristic                        | Cycle 1 (n = 284) | Cycle 2 (n = 571) | Cycle 3 (n = 557) | Cycle 4 (n = 575) |
|---------------------------------------|-------------------|-------------------|-------------------|-------------------|
| Age (yrs); Median (IQR)               | 60.5 (14.0–72.0)  | 60.5 (39.9–71.9)  | 55.5 (39.6–71.7)  | 58.5 (40.9–70.0)  |
| Male, n (%)                           | 160 (56.3)        | 368 (64.4)        | 336 (60.3)        | 348 (60.5)        |
| ICU LOS (days); Median (IQR)          | 3.0 (7.0–7.3)     | 2.6 (1.0–6.8)     | 2.9 (1.8–7.5)     | 2.8 (1.4–6.8)     |
| Hospital LOS (days); Median (IQR)     | 17.0 (7.0–37.9)   | 16.5 (6.9–35.7)   | 16.8 (7.3–34.8)   | 17.7 (6.0–37.2)   |
| Age >65 years, n (%)                  | 112 (39.4)        | 229 (40.1)        | 193 (34.6)        | 195 (33.9)        |
| APACHE II; Median (IQR)               | 20.0 (1.5–26.0)   | 18.0 (13.0–26.0)  | 19.0 (13.0–25.5)  | 18.0 (13.0–24.0)  |
| Admission from community <48 h, n (%)| 145 (51.1)        | 229 (40.1)        | 193 (34.6)        | 195 (33.9)        |
| Admission category: Trauma/surgical, n (%) | 159 (56.0) | 328 (57.4)        | 322 (57.8)        | 313 (54.4)        |
| Medical, n (%)                        | 125 (44.0)        | 243 (42.6)        | 235 (42.2)        | 262 (45.6)        |
| ICU Readmission, n (%)                | 14 (4.9)          | 30 (5.3)          | 23 (4.1)          | 25 (4.3)          |
| Multiple ICU Readmissions, n (%)      | 6 (2.1)           | 15 (2.6)          | 3 (0.5)           | 5 (0.9)           |
| Operative Intervention, n (%)         | 76 (26.8)         | 257 (45.0)        | 246 (44.2)        | 275 (47.8)        |
| Intercranial drain or monitor, n (%)  | 19 (6.7)          | 36 (6.3)          | 31 (5.6)          | 44 (7.7)          |
| Intercostal Drain, n (%)              | 15 (5.3)          | 37 (6.5)          | 38 (6.8)          | 30 (5.2)          |
| Nasogastric catheter, n (%)           | 196 (69.0)        | 4.9 (71.6)        | 426 (76.5)        | 490 (85.2)        |
| Endotracheal Tube or Trachostomy, n (%)| 205 (72.2)       | 391 (68.5)        | 401 (72.0)        | 474 (82.4)        |
| Urinary catheter, n (%)               | 221 (77.8)        | 480 (84.1)        | 486 (87.3)        | 545 (94.8)        |
| Arterial catheter, n (%)              | 215 (75.7)        | 479 (83.9)        | 471 (84.6)        | 521 (90.6)        |
| Central venous catheter, n (%)        | 217 (76.4)        | 383 (67.1)        | 394 (70.7)        | 436 (75.8)        |
| Other vascular (dialytic) catheter, n (%) | 14 (4.9)      | 40 (7.0)          | 44 (7.9)          | 44 (7.7)          |
| Dialysis, n (%)                       | 13 (4.6)          | 23 (4.9)          | 38 (6.8)          | 32 (5.6)          |
| ICU Mortality, n (%)                  | 47 (16.5)         | 66 (11.8)         | 56 (10.1)         | 80 (13.9)         |
| Hospital Mortality, n (%)             | 63 (22.2)         | 72 (12.6)         | 67 (12.0)         | 99 (17.2)         |

*Significant difference between cycle 1 (Unit 1 only) and 2; **between cycle 2 and 3; ***between cycle 3 and 4; ****between cycle 1 and 4; 
*<p>0.05; **p<0.01; ***p<0.001.

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Table 3. MRSA and \( P. \) aeruginosa infection and colonisation after ICU admission.

| Unit | Cycle | Adm. | (rate) | n | (rate; % SI) | n | (rate; % SI) | n/tested | (rate) | n/tested | (rate) |
|------|-------|------|--------|---|--------------|---|--------------|----------|--------|----------|--------|
| 1    | 1 FEP | 180  | 34.44  | 14| 7.78         | 24| 13.33        | 18/51    | 26/51  |
| (n = 743) | 2 APP-β | 181 | 36.46 | 7 | 3.87 | 15 | 8.29 | 21/49 | 23/49 |
| 3 FEP | 182 | 35.71 | 13 | 7.14 | 28 | 15.38* | 23/57 | 23/57 |
| 4 APP-β | 200 | 33.00 | 7 | 3.50 | 18 | 9.00* | 22/52 | 20/52 |
| All FEP | 362 | 35.08 | 27 | (7.46; 21.3) | 52 | (14.36; 40.1) | 41/108 | (38.9) | 49/108 | (48.1) |
| All APP-β | 381 | 34.65 | 14 | (3.67; 10.6) | 33 | (8.66*; 25.0) | 43/101 | (42.6) | 43/101 | (42.6) |
| 2    | 2 APP-β | 144 | 80.15 | 20 | 13.89 | 8 | 5.56 | 6/31 | 0/31 |
| (n = 451) | 3 FEP | 154 | 77.12 | 24 | 15.58 | 8 | 5.19 | 3/26 | 0/26 |
| 4 APP-β | 153 | 55.56* | 13 | 8.50 | 8 | 5.23 | 5/20 | 1/20 |
| All FEP | 154 | 77.12 | 24 | (15.58; 20.3) | 8 | (5.19; 6.8) | 3/26 | (11.5) | 0/26 | (0.0) |
| All APP-β | 297 | 67.50* | 33 | (11.11; 17.5) | 16 | (5.39; 8.5) | 11/51 | (21.6) | 1/51 | (2.0) |

Significant isolates (SI) in patients admitted for more than 48 h (n = 1194) compared with commensal isolates (CI) from those admitted to ICU within 48 h of hospital arrival and received only cefepime or APP-β, or no antibiotics at all, by the end of their first week in ICU (n = 286). FEP, cefepime; APP-β, antipseudomonal penicillin/β-lactamase inhibitor combinations;
*indicates significance (p-value < 0.05) relative to the previous cycle, by Chi-Squared analysis. Rate: patients positive per 100 admissions; % SI: rate per 100 significant isolates.

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Table 4. Antibiotic prescribing trends in cycles.

| Antibiotic | Cycle 1 n (days) | Cycle 2 n (days) | Cycle 3 n (days) | Cycle 4 n (days) |
|------------|------------------|------------------|------------------|------------------|
|            | First            | Mid              | Last             | First            | Mid              | Last             | First            | Mid              | Last             |
| PEN        | 1 (2)            | 0 (0)            | 0 (0)            | 7 (22)           | 8 (56)           | 5 (26)           | 1 (1)           | 8 (28)           | 5 (11)           |
| AMP        | 9 (35)           | 6 (14)           | 9 (22)           | 12 (48)          | 12 (19)          | 13 (39)          | 25 (88)         | 23 (112)         | 23 (60)          |
| CEF/CFZ    | 20 (49)          | 24 (69)          | 17 (31)          | 44 (136)         | 30 (79)          | 46 (121)         | 37 (99)         | 43 (136)         | 41 (115)         |
| FLU/DCX    | 1 (6)            | 5 (21)           | 6 (32)           | 10 (36)          | 9 (34)           | 8 (46)           | 5 (12)          | 9 (53)           | 6 (25)           |
| CAZ        | 0 (0)            | 0 (0)            | 1 (1)            | 1 (3)            | 1 (3)            | 0 (0)            | 3 (3)           | 2 (3)            | 1 (2)            |
|CTX/CRO     | 15 (54)          | 17 (48)          | 14 (47)          | 27 (105)         | 15 (59)          | 15 (42)          | 23 (91)         | 10 (24)          | 15 (51)          |
| FEP        | 21 (133)         | 38 (273)         | 26 (202)         | 5 (31)           | 3 (31)           | 3 (26)           | 68 (409)        | 68 (425)         | 57 (342)         |
| APP-b      | 4 (29)           | 6 (49)           | 6 (23)           | 72 (423)         | 63 (357)         | 70 (346)         | 23 (112)        | 18 (66)          | 15 (75)          |
| MEM        | 7 (29)           | 3 (11)           | 4 (23)           | 21 (167)         | 14 (56)          | 17 (127)         | 11 (65)         | 17 (139)         | 13 (92)          |
| AMK        | 1 (5)            | 1 (4)            | 3 (3)            | 1 (2)            | 1 (2)            | 1 (2)            | 1 (10)          | 0 (0)            | 2 (2)            |
| GEN        | 21 (105)         | 26 (112)         | 23 (70)          | 46 (190)         | 50 (211)         | 38 (167)         | 40 (139)        | 38 (169)         | 55 (269)         |
| TOB        | 0 (0)            | 0 (0)            | 0 (0)            | 6 (46)           | 2 (4)            | 1 (2)            | 1 (2)           | 1 (3)            | 2 (4)            |
| MTZ        | 23 (146)         | 32 (165)         | 28 (102)         | 24 (142)         | 27 (135)         | 21 (91)          | 52 (220)        | 52 (281)         | 49 (215)         |
| CIP        | 3 (15)           | 5 (28)           | 1 (2)            | 13 (114)         | 6 (41)           | 8 (62)           | 8 (32)          | 3 (10)           | 8 (64)           |
| MXF        | 8 (36)           | 7 (57)           | 8 (36)           | 15 (82)          | 2 (12)           | 4 (14)           | 6 (27)          | 9 (43)           | 6 (22)           |
| TEC        | 0 (0)            | 0 (0)            | 0 (0)            | 1 (3)            | 1 (7)            | 1 (5)            | 2 (10)          | 0 (0)            | 0 (0)            |
| VAN        | 26 (122)         | 29 (134)         | 21 (96)          | 53 (333)         | 40 (224)         | 41 (249)         | 45 (243)        | 43 (225)         | 48 (278)         |
| LZD        | 0 (0)            | 0 (0)            | 0 (0)            | 1 (34)           | 0 (0)            | 2 (10)           | 0 (0)           | 0 (0)            | 3 (64)           |
| SXT        | 0 (0)            | 1 (6)            | 0 (0)            | 0 (0)            | 1 (2)            | 1 (5)            | 1 (1)           | 3 (27)           | 2 (18)           |
| CST        | 0 (0)            | 0 (0)            | 0 (0)            | 0 (0)            | 1 (46)           | 0 (0)            | 0 (0)           | 0 (0)            | 0 (0)            |

Number of patients receiving specified antibiotic in each cycle, n, and total patient days (days).
First: first third of cycle; mid: middle third of cycle; last: last third of cycle.
PEN, penicillin; AMP, ampicillin; CEF, cephalothin; CFZ, cefazolin; FLU, flucloxacillin; DCX, dicloxacillin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; APP-b, antipseudomonal penicillin combinations; MEM, meropenem; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; MTZ, metronidazole; CIP, ciprofloxacin; MXF, moxifloxacin; TEC, teicoplanin; VAN, vancomycin; LZD, linezolid; SXT, co-trimoxazole; CST, colistin (polymixin E).
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Figure 3. Antibiotic treatment and *P. aeruginosa* and MRSA colonisation rates. Total defined daily doses of selected antibiotics in cycle thirds in patients admitted to ICU within 48 h of hospital arrival and received only cefepime or APP-b, or no antibiotics at all, by the end of their first week in ICU (vertical bars; left axis), with incidence rates (solid lines; right axis) of unique isolation of MRSA (triangles) and *P. aeruginosa* (squares) from commensal sites (ie. colonisation rates, per 100 admissions).
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is suggested (Figure 3), and many of the relative proportions are significantly different when directly compared (Table 5). Sampling was complete at all time points in 206 of these 286 admissions. Of these 206, 63 (30.6%) developed perineal and/or endotracheal colonisation by antibiotic-resistant bacteria in the first week. Of 63 newly colonised admissions identified, 18 (28.6%) acquired MRSA, 36 (57.1%) acquired P. aeruginosa and 44 (69.8%) acquired resistant Enterobacteriaceae. Backwards stepwise logistic regression analysis revealed cefepime treatment in a cefepime cycle as the sole independent predictor of acquiring resistant bacteria at surveillance sites (OR 2.51; 95% CI 1.35–4.66; p 0.003). Those treated with cefepime were significantly more likely to acquire MRSA (OR 3.612; 95% CI 1.33–9.79; p 0.012) or antibiotic-resistant Enterobacteriaceae (OR 3.184; 95% CI 1.589–6.380; p 0.001). New acquisition of (any) P. aeruginosa was also twice as common in the cefepime as APP-β-treated admissions, but this was not quite statistically significant at 95% confidence levels (OR 2.02; 95% CI 0.96–4.23; p 0.063). The only factor associated with reduced risk of antibiotic-resistant colonization was an operative intervention (OR 0.049; 95% CI 0.241–0.990; p 0.047). Although APP-β treatment did not independently predict reduced risk, cefepime treatment is independently predictive of increased risk of resistance acquisition in multivariate analysis.

### Diversity of MRSA Subtypes

Clonal MRSA outbreaks that were coincidental with the antibiotic cycle changes were excluded as an explanation for these findings. Standardised methodology [22] revealed 35 distinct MRSA pulsortypes, within 17 clearly unrelated groups (<84% similarity; Dice coefficient, represented by UPGMA, 0.5% optimization and 1.0% tolerance) that were distributed throughout the study. The majority of these (~75%) were members of the dominant clonal complex in Australia (Aus 2/3; multi-locus sequence type 239), with complexes 93, 30, 22, 5 and 36 also identified. Diversity was similar in each cycle: cycle 1 (cefepime; Unit 1 only) had 7 PFGE variants of ≤95% similarity, cycle 2 (APP-β) had 10 such variants, cycle 3 (cefepime) had 13 variants and cycle 4 (APP-β) had 11 variants. The largest group of closely related isolates (≥95% similarity) spanned two cycles over a 6-month period (Mar - Sept 2004). This group comprised only 20% of isolates in that period, were interspersed with less closely related isolates, and still occurred sporadically a year later (Figure S1). These data indicate that different MRSA clones appeared throughout the study, as expected for a general selection effect.

### Discussion

The model of antibiotic cycling is part of normal ICU practice, and in similar studies [24,25] a waiver of consent was granted for the same reasons as apply here. Here, we show that cefepime exposure is the strongest single independent risk factor for colonization and infection of intensive care patients by antibiotic-resistant bacteria. We found no evidence of a cumulative or persistent effect, in keeping with a similar TZP/cefepime cycling study in transplant patients that reported preservation of antibiotic susceptibility among Gram-negative bacteria [26].

We found no support for mathematical models that identify antibiotic homogeneity as a specific resistance driver [27]. This conflict may be explained by the necessity to employ simplified assumptions about biological mechanisms [28]. Previous studies of antibiotic cycling often compared antibiotics with vastly different mechanisms of action and ecological effects, and often used extremely short cycles [29] in spite of evidence that antibiotic effects last for many weeks and, for some elements of the microflora, months to years [30]. Activity of cefepime against relevant pathogens was superior to that of APP-β in vitro but APP-β seem to have less adverse effects on the microflora than third-generation cephalosporins [27,30]. Antibiotic levels in tissues or gut were not defined in this study, but biliary penetration of cefepime is relatively poor compared to that of the antipseudomonal penicillins [31]. Unlike cefepime, the activity of antipseudomonal penicillins such as TZP against some of the Enterobacteriaceae with class 1 cephalosporinases is dependent on the tazobactam component, which is relatively less excrated in bile than piperacillin [32], while both gut penetration and the in vitro activity of piperacillin is superior to that of cefepime against the major anaerobic pathogens [31]. Metronidazole (MTZ) was commonly added to cefepime by prescribers in this study and differences in activity (between the cefepime/MTZ combination and the antipseudomonal penicillin/β-lactamase inhibitor combinations) against commensal anaerobes may also be relevant. In the mouse model, disruption of the gut microflora by a third-generation cephalosporin does not spontaneously revert, unlike the effects of a penicillin (ampicillin) [33]. An association between third-generation cephalosporins [34] and cefepime [35] and increased prevalence of resistant pathogens such as Clostridium difficile [36,37], P. aeruginosa [38], antibiotic-resistant Enterobacteriaceae [39] and MRSA [39,40,41] is well described. Lasting effects on the microflora, as reported for other antibiotics [8,9], cannot be excluded in the absence of long-term follow-up after ICU discharge but we found

### Table 5. Colonisation of patients with MRSA or P. aeruginosa within portions of cycles.

|          | MRSA        | P. aeruginosa |
|----------|-------------|---------------|
|          | FEP         | APP-β         | FEP          | APP-β         | FEP          | APP-β         |
|          | n/tested    | rate          | p n/tested   | rate          | p n/tested   | rate          |
| First Half | 21/68       | 0.31          | 0.076       | 24/70         | 0.34         | 0.211         | 0.32          | 0.006         | 0.46          | 0.18          |
| Second Half | 31/66       | 0.47          | 0.24        | 30/66         | 0.45         | 0.018         | 0.33          | 0.21          | 0.32          | 0.023         |
| First Third | 16/50       | 0.32          | 0.45        | 14/50         | 0.28         | 0.192         | 0.51          | 0.21          | 0.32          | 0.023         |
| Second Third | 17/42      | 0.4           | 0.49        | 14/42         | 0.33         | 0.192         | 0.51          | 0.21          | 0.32          | 0.023         |
| Last Third  | 19/42       | 0.45          | 0.4         | 11/49         | 0.22         | 0.192         | 0.51          | 0.21          | 0.32          | 0.023         |

P-values indicate significance between portions of cycles for each organism, by Chi-Squared analysis. FEP, cefepime; APP-β, antipseudomonal penicillin/β-lactamase inhibitor combinations. Rate: proportion of patients positive.

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Table 6. Infection rates.

| Source | Organism | Unit 1 Rate | Unit 2 Rate | p* |
|--------|----------|-------------|-------------|----|
| blood  | MRSA     | 6           | 1.2         |    |
|        | MSSA     | 6           | 1.2         |    |
|        | Enterococcus | 8     | 3.5         |    |
|        | Streptococcus | 3   | 2.6         |    |
|        | CNS      | 89         | 37          | 0.74 |
|        | E. coli  | 3          | 1.2         |    |
|        | Klebsiella | 2    | 1.8         |    |
|        | ESCPM b  | 5          | 3.5         |    |
|        | P. aeruginosa | –  | 2.4         |    |
| bacterial total | 122 | 49          | 57          |    |
| (excl. CNS) | 35   | 12          | 14.1        | 0.022 |
| fluids and tissues c | MRSA | 2          | –           | –  |
|        | MSSA     | 2          | 1.8         | –  |
|        | Enterococcus | –    | 1.2         | –  |
|        | CNS      | 14         | 17          | 20  |
|        | E. coli  | 4          | 3.5         | 3.5 |
|        | Klebsiella | 1   | 0.9         | 2.4 |
|        | ESCPM b  | –          | 4           | 4.7 |
|        | P. aeruginosa | 5    | 3.5         |    |
| bacterial total | 29   | 30          | 36.2        |    |
| (excl. CNS) | 15   | 13          | 15.3        | 0.075 |
| urine  | MRSA     | –          | –           | –  |
|        | MSSA     | –          | 1.2         |    |
|        | Enterococcus | 9    | 2.4         |    |
|        | CNS      | –          | 1.2         |    |
|        | E. coli  | 8          | 9.4         |    |
|        | Klebsiella | 4   | 2.4         |    |
|        | ESCPM b  | 7          | 3           | 3.5 |
|        | P. aeruginosa | 8   | 4.7         |    |
| bacterial total | 37   | 22          | 25.8        |    |
| (excl. CNS) | 37   | 21          | 24.6        | 0.347 |
| respiratory | MRSA    | 40         | 8           | 9.4 |
|        | MSSA     | 24         | 53          | 62.2 |
|        | Enterococcus | –    | 1.2         | –  |
|        | S. pneumoniae | –    | 5.9         |    |
|        | E. coli  | 11         | 21          | 24.6 |
|        | Klebsiella | 19  | 49          | 57  |
|        | ESCPM b  | 28         | 39          | 45.8 |
|        | P. aeruginosa | 32  | 32.9        |    |
| bacterial total | 165  | 248         | 314.6       |    |
| (excl. NF) | 154  | 221         | 259.4       | <0.001 |

Unique infections in all patients admitted to ICU (n = 1987) are shown in absolute numbers and in rates and a two-tailed test applied to compare the rates per 1000 admissions.

Table 6. Cont.

*Includes surgical specimens and drainage procedures;

dNF: glucose non-fermenting Gram-negative bacilli, including Acinetobacter spp., Pseudomonas spp. Stenotrophomonas spp. etc.

**Chi-Squared analysis.

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no evidence of cumulative risk. Antibiotic resistance rates overall fell to baseline in APP-β cycles, presumably as cephepine-exposed admissions were discharged from ICU. MRSA and P. aeruginosa infection rates also rose and fell according to cycle, and underlying colonisation rates appeared to behave in the same way.

Cefepime was associated with increased P. aeruginosa colonisation and infection, and significantly so for combined FEP cycles (27/362) vs APP-β cycles (14/381) in Unit 1 (p 0.024). Ratios between bacteraemia and respiratory infection and deep isolates were similar between Units for MRSA and for methicillin-sensitive S. aureus (MSSA), although respiratory infection was more commonly reported in Unit 2 in general, and this included Gram-negative pathogens as well as S. aureus (Table 6). Indeed, the overwhelming source of all significant isolates in Unit 2 was respiratory, at which site the attribution of significance to pathogens such as MRSA and P. aeruginosa is problematic. Nevertheless, rates of infection in fluids and tissues were similar between Units, as expected given the similar casemix and acuity. More frequent reporting of P. aeruginosa in Unit 2 (a burns referral centre) may have arisen indirectly, through greater awareness of the pathogen, and this would be another potential confounder. Any bias introduced by relatively higher incidence of community admissions to Unit 2 introduces would be expected to operate to reduce rates of nosocomial Pseudomonas infection but Unit 2 also reported a higher rate of urinary catheterization which, combined with the possible variation in respiratory tract reporting, may relatively increase apparent Pseudomonas infection rates. In any case however, the fact that infection was less common in Unit 2 than Unit 1 in almost every site including the bloodstream and that only Unit 2 experienced P. aeruginosa bacteraemias suggests that the difference is real. This is difficult to further clarify, although it is reasonable to assume that any attribution of undue significance to respiratory P. aeruginosa in Unit 2 and/or under-attribution in Unit 1 would be unaffected by cycling and remain relatively constant from cycle to cycle.

Neither length of stay nor mortality changed significantly overall in this study, despite the known consequences of antibiotic-resistant infection [42]. In Unit 1, with the highest MRSA incidence, MRSA infection complicated 8.7% of APP-β cycle admissions, with more than a 60% increase (to 14.4%) in cefepime cycle admissions. If MRSA infection doubles mortality risk [43], a cohort of at least 1000 cefepime-treated patients would be required to detect a difference over a baseline mortality of 12%. A meta-analysis of 57 studies comparing cefepime with another β-lactam antibiotic in more than 3000 patients with sepsis reported an increased risk of all-cause mortality associated with cefepime not long after this study closed recruiting, the greatest difference being between TZP and FEP [12,44]. These findings were later disputed [45] and then contradicted [46], but discrepancies between the original data and the unpublished data submitted by the drug sponsor [47] left the issue unresolved [48,49]. Longer cycles or a larger study might have revealed statistically stronger differences. A disproportionate incidence of MRSA in Unit 1 and the absence of a second cefepime cycle in Unit 2 are weaknesses, but clonal MRSA outbreaks in Unit 1 do
not provide an explanation. Overall antibiotic resistance was not detectably increased in our study by antibiotic cycling and independently obtained data from NSW Health also showed that MRSA acquisition rates did not increase in Unit 1 after the cycling study (Figure S2).

Our data show that cefepime therapy is associated with increased infection due to organisms resistant to the key antibiotics used for management of sepsis and septic shock in intensive care. Importantly, prescribing homogeneity per se does not appear to be a specific resistance driver. The ecological influences of cefepime seem to exceed that of all other risk factors in determining infection risk and may be relevant to unexplained mortality differences between cefepime and TZP in large studies of the critically ill. Failure to consider ecological effects in setting antibiotic policy may increase antibiotic resistance and even contribute to unintended mortality.

Supporting Information

Figure S1 Temporal distribution of MRSA pulsotypes within cycling period. ST: Sequence type (letters indicate subgroups within STs at 95% identity). FEP: cefepime cycles; APP-β: antipseudomonal penicillin combination cycles. (TIF)

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