Antimicrobial Resistance in Invasive Bacterial Infections in Hospitalized Children, Cambodia, 2007–2016

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To determine trends, mortality rates, and costs of antimicrobial resistance in invasive bacterial infections in hospitalized children, we analyzed data from Angkor Hospital for Children, Siem Reap, Cambodia, for 2007–2016. A total of 39,050 cultures yielded 1,341 target pathogens. Resistance rates were high; 82% each of *Escherichia coli* and *Klebsiella pneumoniae* isolates were multidrug resistant. Hospital-acquired isolates were more often resistant than community-acquired isolates; resistance trends over time were heterogeneous. *K. pneumoniae* isolates from neonates were more likely than those from nonneonates to be resistant to ampicillin–gentamicin and third-generation cephalosporins. In patients with community-acquired gram-negative bacteremia, third-generation cephalosporin resistance was associated with increased mortality rates, increased intensive care unit admissions, and 2.26-fold increased healthcare costs among survivors. High antimicrobial resistance in this setting is a threat to human life and the economy. In similar low-resource settings, our methods could be reproduced as a robust surveillance model for antimicrobial resistance.

Worldwide, invasive bacterial infections are a leading cause of childhood deaths, mostly in low- and middle-income countries (*1*). Management of such infections is threatened by the rising prevalence of antimicrobial resistance (AMR), particularly among neonates (*2*). However, data on AMR in invasive bacterial infections in children from low- and middle-income countries are scarce (*3*–*6*).

To combat the global threat of AMR, improved surveillance to detect emerging and long-term resistance trends is vital (*7*). Several global initiatives, such as the Fleming Fund, have been recently established to improve laboratory capacity in low- and middle-income countries (*7*,*8*), and the World Health Organization (WHO) Global Antimicrobial Resistance Surveillance System (GLASS) (*9*) has targeted 6 invasive pathogens for routine antimicrobial resistance surveillance: *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Salmonella* spp., *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Monitoring resistance in these pathogens is particularly important for invasive bacterial infections in children in low- and middle-income countries, where most treatment is empirically prescribed and must be based on reliable contemporaneous resistance data to be effective.

Recent systematic reviews of AMR in invasive bacterial infections in children highlight the paucity of data available and do not report temporal resistance trends (*5*,*6*). In addition, although recent studies indicate excess deaths caused by AMR in low- and middle-income countries (*10*), there is limited evidence describing the economic and mortality burden of resistance at the patient level, particularly among children.

We analyzed 10 years of continuous AMR surveillance data for invasive bacterial infections in children from a sentinel surveillance site in Cambodia and describe resistance trends over time, by age group, and by site of acquisition (community or hospital). To evaluate the excess deaths and cost burden associated with third-generation cephalosporin resistance in community-acquired gram-negative bacteremia in hospitalized children, we analyzed patient-level data.

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Methods

Study Design and Sample Selection

Angkor Hospital for Children is an ≈100-bed nongovernmental hospital in Siem Reap, Cambodia. Of children admitted, around two thirds reside in Siem Reap Province (*11*), where the incidence of poverty exceeds 50% (*12*); 93% are admitted from the community and 7% are transferred from another hospital (P. Turner, unpub data). Because this hospital has no maternity/obstetric ward, all children are born outside the hospital. Blood cultures are routinely taken from febrile (axillary temperature >37.5°C) hospitalized patients, according to clinical algorithms, at no patient cost.

We reviewed hospital microbiology data for 2007–2016 and extracted AMR data for selected blood culture and cerebrospinal fluid (CSF) culture isolates. Target organisms consisted of the 6 GLASS blood culture priority pathogens (*9*), *Neisseria meningitidis* (a vaccine-preventable pathogen), and non-GLASS pathogens for which ≥30 organisms were isolated over the study period. We included in the study the first isolate of a given organism per patient per 14-day infection episode, except for *Salmonella* spp., for which we included only the first isolate per patient to avoid double counting potential relapses. Clinical data were extracted from hospital patient records. The study was approved by the Angkor Hospital for Children Institutional Review Board (AHC-IRB, 0185-17) and the Oxford Tropical Research Ethics Committee (OxTREC, 508-17).

Procedures and AMR Reporting

We processed blood and CSF culture specimens as described elsewhere (*11*,*13*). Antimicrobial susceptibility testing was undertaken by disk diffusion and Etest MIC, according to Clinical and Laboratory Standards Institute guidelines (*14*) (online Technical Appendix Methods and Table 1, https://wwwnc.cdc.gov/EID/article/24/5/17-1830-Techapp1.pdf). Resistance proportions are reported as number of resistant isolates/number of isolates tested.

Outcome Analyses

We included in patient outcome analyses community-acquired monomicrobial *Enterobacteriaceae* (excluding *Salmonellae*) and *A. baumannii* bacteremia. These pathogens represent common causes of sepsis in children worldwide where third-generation cephalosporins would be a first-line/empiric treatment. We obtained clinical and costing data from hospital records and calculated cost per patient as admission cost plus antimicrobial costs.

Statistical Analyses

We treated isolates from specimens taken within 48 hours of admission as community-acquired infections and after 48 hours as hospital-acquired infections. However, *Salmonella enterica* serotypes Typhi and Paratyphi and *Burkholderia pseudomallei* isolates were always considered community-acquired infections. To ensure sufficient data per period, we grouped isolates into 2-year blocks. We assessed associations between resistance and year of isolation, patient age group, and site of acquisition (community vs. hospital) by univariable and multivariable logistic regression. Multivariable models included all variables. According to assessment of model fit by calculation of Akaike information criterion and plotting of observed versus predicted data, we considered time (year of isolation) a factor unless otherwise stated.

For the outcome analyses, we used univariable analysis to compare variables by third-generation cephalosporin resistance status and patient outcome by using the Mann-Whitney-Wilcoxon rank-sum test for continuous variables and the χ^2 test with Yates correction for categorical variables. For multivariable logistic regression, outcome variables were hospital deaths and intensive care unit (ICU) admissions, and covariates were resistance, age group, age <10 years, malnutrition, sex, and organism type (*Enterobacteriaceae* vs. *A. baumannii*). We conducted multivariable linear regression by using admission duration and cost for survivors as outcome variables and using the same covariates. The linear model variables were log transformed, and results are presented with log- and back-transformed coefficients, which is interpreted as a multiplicative rather than an additive model. Analyses were undertaken by using the R statistical package (*15*).

Results

During the 10-year study period, 39,050 sterile site samples were collected for culture: 36,358 (93.1%) blood and 2,692 (6.9%) CSF (online Technical Appendix Figure 1). The sampling rate, indicated by the blood culture:hospital admission ratio, rose throughout the study period as utility of the clinical microbiology service increased. Approximately 1 blood culture was sent for every 3 admissions in 2007 (1,293 blood cultures:3,829 admissions), rising to 1 blood culture per admission in 2013 (5,294 blood cultures:5,208 admissions) and subsequently remaining stable (online Technical Appendix Figure 2). From 2012 through 2016, the proportion of blood cultures from neonates rose from 9.1% to 21.2%, and the proportion from children ≥5 years of age dropped from 35.4% to 22.4% (online Technical Appendix Table 2).

Of the 39,050 specimens collected, 3,666 (9.4%) were culture positive, yielding 4,028 isolates. Skin organism contamination was identified in 1,937 (5.3%) blood cultures. Clearly pathogenic bacteria comprised 37.5% (1,512) of isolates grown, 9.1% (366) were of uncertain significance,

and 53.4% (2,150) were designated skin contaminants. A total of 1,341 target organisms met inclusion criteria; 1,088 (81.1%) were GLASS pathogens and 253 (18.9%) were non-GLASS pathogens. GLASS pathogens were *Salmonella* spp. (408, 30.4%); *S. aureus* (186, 13.9%); *S. pneumoniae* (166, 12.4%); *K. pneumoniae* (146, 10.9%); *E. coli* (107, 8.0%); and *A. baumannii* (75, 5.6%).

Overall AMR Rates

Overall AMR rates were high, especially among gram-negative GLASS organisms (Table 1). Ampicillin–gentamicin resistance (resistance to both agents) was detected in 62.1% (90/145) of *K. pneumoniae* isolates and 47.2% (50/106) of *E. coli* isolates. Third-generation cephalosporin resistance was detected in 78.8% (115/146) of *K. pneumoniae* isolates, 49.5% (53/107) of *E. coli* isolates, and 93.3% (70/75) of *A. baumannii* isolates; multidrug resistance in these 3 organisms was 81.8% (108/132), 82.1% (69/84), and 93.3% (70/75), respectively. Carbapenem resistance was uncommon: <1% of *K. pneumoniae* (1/142) and *E. coli* isolates (0/98) and 13.5% (10/74) of *A. baumannii* isolates were resistant.

Resistance differed greatly among the 3 groups of *Salmonella* spp. The proportion of resistant isolates was highest for *Salmonella* Typhi: 95.7% (308/322) were fluoroquinolone resistant, 86.0% (270/314) multidrug resistant, and 85.0% (266/313) fluoroquinolone and multidrug resistant. The least resistant group was *Salmonella* Paratyphi A: 22.7% (10/44) of isolates were fluoroquinolone resistant and none were multidrug resistant (0/43).

Table 1. Resistance proportions by year of isolation for the 1,088 Global Antimicrobial Resistance Surveillance System pathogens isolated from children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016*

Pathogen, resistance type	No. isolates resistant/no. tested (%)	Year of isolation				
		2007–2008	2009–2010	2011–2012	2013–2014	2015–2016
Gram-negative						
Klebsiella pneumoniae	146	11	17	56	42	20
AMP–GEN†	90/145 (62.1)	5/11 (45.5)	10/16 (62.5)	46/56 (82.1)	26/42 (61.9)	3/20 (15.0)
3GC	115/146 (78.8)	8/11 (72.7)	13/17 (76.5)	50/56 (89.3)	37/42 (88.1)	7/20 (35.0)
Carbapenem	1/142 (0.7)	0/8	0/16	0/56	1/42 (2.4)	0/20
Multidrug	108/132 (81.8)	8/8 (100)	12/12 (100)	45/50 (90.0)	36/42 (85.7)	7/20 (35.0)
Escherichia coli	107	12	22	21	30	22
AMP–GEN	50/106 (47.2)	4/12 (33.3)	12/21 (57.1)	8/21 (38.1)	17/30 (56.7)	9/22 (40.9)
AMP	101/107 (94.4)	10/12 (83.3)	21/22 (95.5)	21/21 (100)	28/30 (93.3)	21/22 (95.5)
GEN	51/106 (48.1)	4/12 (33.3)	12/21 (57.1)	8/21 (38.1)	18/30 (60.0)	9/22 (40.9)
3GC	53/107 (49.5)	3/12 (25.0)	11/22 (50.0)	11/21 (52.4)	16/30 (53.3)	12/22 (54.5)
Carbapenem	0/98	0/3	0/22	0/21	0/30	0/22
Multidrug	69/84 (82.1)	3/3 (100)	13/13 (100)	15/16 (93.8)	23/30 (76.7)	15/22 (68.2)
Acinetobacter baumannii	75	2	7	30	27	9
3GC	70/75 (93.3)	2/2 (100)	6/7 (85.7)	27/30 (90.0)	27/27 (100)	8/9 (88.9)
Carbapenem	10/74 (13.5)	1/2 (50.0)	1/6 (16.7)	5/30 (16.7)	3/27 (11.1)	0/9
Multidrug	21/71 (29.6)	1/2 (50.0)	2/6 (33.3)	9/27 (33.3)	8/27 (29.6)	1/9 (11.1)
Salmonella Typhi	323	44	51	146	40	42
FQ	308/322 (95.7)	39/44 (88.6)	48/51 (94.1)	139/145 (95.9)	40/40 (100)	42/42 (100)
CRO	1/173 (0.6)	0/44	1/21 (4.8)	0/26	0/40	0/42
MDR	270/314 (86.0)	31/41 (75.6)	39/47 (83.0)	134/144 (93.1)	35/40 (87.5)	31/42 (73.8)
FQ and multidrug	266/313 (85.0)	30/41 (73.2)	38/47 (80.9)	132/143 (92.3)	35/40 (87.5)	31/42 (73.8)
Salmonella Paratyphi A	44	3	0	0	35	6
FQ	10/44 (22.7)	3/3 (100)			4/35 (11.4)	3/6 (50.0)
CRO	0/44	0/3			0/35	0/6
MDR	0/43	0/2			0/35	0/6
FQ and multidrug	0/0	0/0			0/0	0/0
Non-Typhoid *Salmonellae*	41	7	4	7	9	14
FQ	26/41 (63.4)	4/7 (57.1)	2/4 (50.0)	4/7 (57.1)	6/9 (66.7)	10/14 (71.4)
CRO	3/37 (8.1)	0/7	0/4	1/3 (33.3)	0/9	2/14 (14.3)
Multidrug	9/39 (23.1)	3/7 (42.9)	1/2 (50.0)	2/7 (28.6)	2/9 (22.2)	1/14 (7.1)
FQ and multidrug	5/39 (12.8)	3/7 (42.9)	1/2 (50.0)	0/7	0/9	1/14 (7.1)
Gram-positive						
Staphylococcus aureus	186	26	38	43	42	37
MET	24/185 (13.0)	3/26 (11.5)	4/38 (10.5)	8/42 (19.0)	3/42 (7.1)	6/37 (16.2)
VAN	0/9	0/0	0/0	0/0	0/3	0/6
Streptococcus pneumoniae	166	17	36	40	41	32
Penicillin	73/144 (50.7)	5/9 (55.6)	10/23 (43.5)	16/39 (41.0)	20/41 (48.8)	22/32 (68.8)
MAC/LIN	49/165 (29.7)	5/17 (29.4)	10/35 (28.6)	12/40 (30.0)	11/41 (26.8)	11/32 (34.4)
MDR	63/93 (67.7)	0/0	0/0	10/20 (50.0)	26/41 (63.4)	27/32 (84.4)

*Resistance proportions are reported as no. resistant isolates/no. isolates tested. Blank cells indicate that no organisms were tested during that period and, thus, the proportion of resistant organisms is unknown. 3GC, third-generation cephalosporin; AMP–GEN, resistance to both ampicillin and gentamicin; CRO, ceftriaxone; FQ, fluoroquinolone; MDR, multidrug resistant; MAC/LIN, resistance to macrolides and/or lincosamides; MET, methicillin; VAN, vancomycin.
†*K. pneumoniae* is intrinsically resistant to AMP, and thus AMP–GEN resistance in *K. pneumoniae* isolates is equivalent to GEN resistance.

Resistance in nontyphoidal *Salmonella* spp. fell between that of *Salmonella* Typhi and Paratyphi A: 63.4% (26/41) of isolates were fluoroquinolone resistant, 23.1% (9/39) multidrug resistant, and 12.8% (5/39) fluoroquinolone and multidrug resistant. Only 1.6% (4/254) of *Salmonella* spp. isolates were ceftriaxone resistant.

In gram-positive GLASS organisms, approximately one third of *S. pneumoniae* isolates were macrolide/lincosamide resistant (29.7%, 49/165), half were penicillin resistant (50.7%, 73/144), and two thirds were multidrug resistant (67.8%, 63/93). Only 13.0% (24/185) of *S. aureus* isolates were methicillin resistant.

The most frequently isolated non-GLASS pathogen was *Burkholderia pseudomallei*, the causative agent of melioidosis (26.1%, 66), which was universally sensitive to the first-line drugs ceftazidime and co-trimoxazole (Table 2). Next was *Haemophilus influenzae*, for which approximately half of isolates were ampicillin resistant (53.6%, 30/56) and one third multidrug resistant (37.1%, 13/35), followed by *Enterobacter cloacae*, which had a similar resistance profile to *K. pneumoniae* and *E. coli*. The remaining non-GLASS pathogens (group A *Streptococcus*, *Pseudomonas aeruginosa*, and *Neisseria meningitides*) exhibited low-level resistance to the key antimicrobials reported.

AMR Time Trends

The most frequently isolated organisms were *K. pneumoniae, E. coli, Salmonella* Typhi, *S. aureus*, and *S. pneumoniae;* AMR time trends were heterogeneous (Tables 1, 3–5; Figure 1). *S. pneumoniae* penicillin resistance fluctuated over time; 55.6% (5/9) of isolates were resistant in 2007–2008, dropping to 41.0% (16/39) in 2011–2012 before rising to 68.8% (22/32) in 2015–2016. During 2011–2016, when we tested *S. pneumoniae* for multidrug resistance, the proportion of multidrug-resistant *S. pneumoniae* isolates increased from 50.0% to 84.4%. *Salmonella* Typhi fluoroquinolone resistance also increased over the study period, from 88.6% to 100%. Multivariable logistic regression analysis in which time was a continuous variable showed an increased probability of *Salmonella* Typhi fluoroquinolone resistance over time (adjusted odds ratio [aOR] 2.14, 95% CI 1.29–3.74; p = 0.005), although not statistically significant in the model when time was a factor (online Technical Appendix Table 3 and Figure 3). Conversely, during 2015–2016, the proportion of resistant *K. pneumoniae* isolates fell dramatically for most antimicrobials tested, a phenomenon not seen for *E. coli* (online Technical Appendix Table 4). For *E. coli*, ampicillin–gentamicin and third-generation cephalosporin resistance remained stable at ≈50% with no evidence of significant change over time, as did rates of methicillin-resistant *S. aureus*, which remained low throughout the study period. The proportion of *K. pneumoniae* isolates from neonates peaked in 2011–2014 at 46%–50% before dropping in 2015–2016 to 35% (online Technical Appendix Table 5), paralleling the change in the proportion of resistant isolates seen. To determine any subtle shifts in susceptibility, we examined changes in zone diameter distribution over time for *E. coli* and *K. pneumoniae* and found no clear trends (online Technical Appendix Table 6, Figures 4, 5).

Table 2. Resistance proportions by year of isolation for the 253 non-Global Antimicrobial Resistance Surveillance System pathogens isolated from children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016*

Pathogen, resistance type	No. isolates resistant/no. tested (%)	Year of isolation				
		2007–2008	2009–2010	2011–2012	2013–2014	2015–2016
Burkholderia pseudomallei	66	6	10	13	22	15
CAZ	0/66	0/6	0/10	0/13	0/22	0/15
TMP/SXT	0/61	0/2	0/10	0/12	0/22	0/15
Haemophilus influenzae	57	15	15	9	12	6
AMP	30/56 (53.6)	5/14 (35.7)	10/15 (66.7)	7/9 (77.8)	8/12 (66.7)	0/6
CRO	3/57 (5.3)	1/15 (6.7)	1/15 (6.7)	0/9	1/12 (8.3)	0/6
Multidrug	13/35 (37.1)	0/0	5/10 (50.0)	5/7 (71.4)	3/12 (25.0)	0/6
Enterobacter cloacae	42	2	6	8	17	9
AMP–GEN	19/42 (45.2)	1/2 (50.0)	5/6 (83.3)	5/8 (62.5)	6/17 (35.3)	2/9 (22.2)
3GC	34/42 (81.0)	1/2 (50.0)	5/6 (83.3)	7/8 (87.5)	14/17 (82.4)	7/9 (77.8)
Carbapenem	3/41 (7.3)	0/1	0/6	0/8	2/17 (11.8)	1/9 (11.1)
Multidrug	18/37 (48.6)	1/1 (100)	2/2 (100)	5/8 (62.5)	7/17 (41.2)	3/9 (33.3)
Group A *Streptococcus*	38	2	6	6	13	11
MAC/LIN	6/37 (16.2)	0/2	1/5 (20.0)	0/6	2/13 (15.4)	3/11 (27.3)
Pseudomonas aeruginosa	37	7	6	7	9	8
CAZ	4/34 (11.8)	0/4	1/6 (16.7)	1/7 (14.3)	2/9 (22.2)	0/8
Carbapenem	2/30 (6.7)	0/1	0/5	1/7 (14.3)	0/9	1/8 (12.5)
Multidrug	0/29	0/0	0/5	0/7	0/9	0/8
Neisseria meningitidis	13	6	3	0	2	2
CRO	1/13 (7.7)	0/6	1/3 (33.3)		0/2	0/2

*Resistance proportions have been reported as number of resistant isolates out of number of isolates tested. Blank cell indicates that no organisms were tested during that period and, thus, the proportion of resistant organisms is unknown. 3GC, third-generation cephalosporin; AMP–GEN, resistance to both ampicillin and gentamicin; CAZ, ceftazidime; CRO, ceftriaxone; MAC/LIN, resistance to macrolides and/or lincosamides; TMP/SXT, trimethoprim/sulfamethoxazole.

AMR by Patient Age Group

Isolates from younger children were more often resistant to clinically important antimicrobials (Tables 3–5; Figure 2; online Technical Appendix Tables 7–9). Multivariable logistic regression controlling for year of isolation and site of acquisition indicated that *K. pneumoniae* isolates from neonates were >7 times more likely to be resistant to the first-line treatment agents ampicillin–gentamicin

Table 3. Logistic regression analysis of resistance trends for the gram-negative Global Antimicrobial Resistance Surveillance System pathogens *Klebsiella pneumoniae* and *Escherichia coli* isolated from children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016*

Pathogen, resistance type, predictor variable	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Klebsiella pneumoniae				
AMP–GEN				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	2.00 (0.42–10.03)	0.384	1.31 (0.23–7.88)	0.765
2011–2012	5.52 (1.41–22.90)	0.015	2.61 (0.58–12.45)	0.213
2013–2014	1.95 (0.51–7.80)	0.329	0.59 (0.12–2.85)	0.504
2015–2016	0.21 (0.03–1.12)	0.075	0.06 (0.01–0.41)	0.006
Patient age				
Nonneonate	Ref	Ref	Ref	Ref
Neonate†	5.63 (2.61–13.10)	<0.001	7.30 (2.75–22.47)	<0.001
Infection type‡				
Community-acquired	Ref	Ref	Ref	Ref
Hospital-acquired	3.87 (1.81–8.51)	<0.001	3.62 (1.42–9.58)	0.008
3GC				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	1.22 (0.20–7.02)	0.823	0.87 (0.13–5.60)	0.881
2011–2012	3.13 (0.57–14.69	0.156	1.37 (0.23–7.16)	0.716
2013–2014	2.78 (0.49–13.93)	0.218	0.97 (0.15–5.58)	0.973
2015–2016	0.20 (0.03–0.94)	0.052	0.06 (0.01–0.39)	0.005
Patient age				
Nonneonate	Ref	Ref	Ref	Ref
Neonate	6.41 (2.32–22.70)	0.001	7.50 (2.16–35.00)	0.004
Infection type				
Community-acquired	Ref	Ref	Ref	Ref
Hospital-acquired	4.04 (1.76–9.44)	0.001	3.51 (1.27–10.12)	0.017
Escherichia coli				
AMP–GEN				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	2.67 (0.63–12.75)	0.194	2.86 (0.66–14.13)	0.174
2011–2012	1.23 (0.28–5.86)	0.785	0.95 (0.20–4.75)	0.947
2013–2014	2.62 (0.67–11.63)	0.179	2.25 (0.53–10.72)	0.282
2015–2016	1.38 (0.33–6.50)	0.665	1.03 (0.22–5.11)	0.975
Patient age				
Nonneonate	Ref	Ref	Ref	Ref
Neonate	1.04 (0.42–2.57)	0.924	0.75 (0.27–2.01)	0.568
Infection type				
Community-acquired	Ref	Ref	Ref	Ref
Hospital-acquired	2.33 (1.04–5.33)	0.041	2.92 (1.21–7.44)	0.020
3GC				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	3.00 (0.68–16.38)	0.165	4.04 (0.79–26.54)	0.112
2011–2012	3.30 (0.74–18.21)	0.134	2.47 (0.45–16.60)	0.319
2013–2014	3.43 (0.83–17.83)	0.105	3.07 (0.60–19.99)	0.201
2015–2016	3.60 (0.82–19.73)	0.106	2.44 (0.45–16.13)	0.319
Patient age				
Nonneonate	Ref	Ref	Ref	Ref
Neonate	0.92 (0.37–2.27)	0.861	0.41 (0.12–1.26)	0.131
Infection type				
Community-acquired	Ref	Ref	Ref	Ref
Hospital-acquired	7.50 (3.09–20.01)	<0.001	10.14 (3.70–32.14)	<0.001

*3GC, third-generation cephalosporin; AMP–GEN, resistance to both ampicillin and gentamicin; OR, odds ratio; ref, referent.
†Neonate, 0–28 d of age.
‡Isolates were defined as hospital-acquired if taken >48 hours after admission.

Table 4. Logistic regression analysis of resistance trends for the gram-negative Global Antimicrobial Resistance Surveillance System pathogen *Salmonella enterica* serovar Typhi isolated from children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016*

Resistance type, predictor variable	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Fluoroquinolone				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	2.05 (0.47–10.51)	0.345	1.85 (0.42–9.59)	0.422
2011–2012	2.97 (0.82–10.37)	0.085	3.05 (0.83–10.74)	0.080
2013–2014	4.03×10^7 (6.26×10^{45}–∞)	0.992	3.47×10^7 (9.49×10^{-44}–∞)	0.992
2015–2016	4.03×10^7 (1.11×10^{-43}–∞)	0.992	4.14×10^7 (6.26×10^{-43}–∞)	0.991
Patient age, y				
≥5	Ref	Ref	Ref	Ref
<5	4.48 (0.87–82.12)	0.151	4.57 (0.87–84.30)	0.150
Multidrug				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	1.57 (0.56–4.58)	0.395	1.45 (0.50–4.29)	0.491
2011–2012	4.32 (1.64–11.44)	0.003	4.55 (1.71–12.17)	0.002
2013–2014	2.26 (0.72–7.92)	0.175	2.08 (0.65–7.41)	0.228
2015–2016	0.91 (0.33–2.46)	0.850	0.95 (0.34–2.62)	0.927
Patient age, y				
≥5	Ref	Ref	Ref	Ref
<5	2.94 (1.22–8.79)	0.029	3.16 (1.28- 9.57)	0.022

*OR, odds ratio; ref, referent.

(aOR 7.30, 95% CI 2.75–22.47) and third-generation cephalosporins (aOR 7.50, 95% CI 2.16–35.00) than were isolates from nonneonates. Similarly, *S. pneumoniae* isolates were more likely to be penicillin resistant (aOR 3.87, 95% CI 1.77–8.83) and *Salmonella* Typhi isolates more likely to be multidrug resistant (aOR 3.16, 95% CI 1.28-9.57) among children <5 years of age than among those ≥5 years of age.

AMR by Site of Infection Acquisition

Approximately four fifths of included isolates were from community-acquired infections (1,089, 81.2%) and one fifth from hospital-acquired infections (252, 18.8%). In almost all instances, the proportion of hospital-acquired isolates resistant to a given antimicrobial was higher than that of community-acquired isolates (online Technical Appendix Tables 10–12). *K. pneumoniae*, the main cause of hospital-acquired infections, was >3 times more likely to be resistant to ampicillin–gentamicin (aOR 3.62, 95% CI 1.42–9.58) and third-generation cephalosporins (aOR 3.51, 95% CI 1.27–10.12) in hospital-acquired isolates (Tables 3, 4). Increased likelihood of resistance among hospital-acquired isolates was also found for *E. coli* ampicillin–gentamicin and third-generation cephalosporin resistance and *S. aureus* methicillin resistance.

Outcomes

We analyzed patient outcomes for 129 admission episodes for community-acquired monomicrobial gram-negative bacteremia (online Technical Appendix Figure 6). Of these, 63 (48.8%) isolates were resistant to third-generation cephalosporins and 34 admissions (26.4%) resulted in patient death. Isolates consisted of *E. coli* (48, 37.2%), *K. pneumoniae* (31, 24.0%), *A. baumannii* (29, 22.5%), and other pathogenic *Enterobacteriaceae* (21, 16.3%). Neonates accounted for 26.4% (34) of the cases; median age was 8.6 months (interquartile range [IQR] 0.8–29.2 months).

Children from whom third-generation cephalosporin-resistant bacteria were isolated were less likely than other patients to have received appropriate antimicrobial therapy (57% vs. 94%; p<0.001). If appropriate therapy was received, it was initiated later for children infected with third-generation–resistant than third-generation–sensitive organisms (2 days vs. 0 days after admission for those who survived [p<0.001]; 0.5 days vs. 0 days for those who died [p = 0.004]). Patients who died were younger (median age 1.4 vs. 10.2 months; p = 0.002), were more likely to have been admitted to an ICU (88% vs. 27%; p<0.001), stayed for a shorter time in hospital (3 vs. 8 days; p<0.001), and were more likely to have been infected with *Enterobacteriaceae* than *A. baumannii* (97% vs. 71%; p = 0.003) (online Technical Appendix Table 13). *A. baumannii* infections were associated with high levels of third-generation cephalosporin resistance (90%) but a low mortality rate (3%) despite only 48% of patients having received appropriate antimicrobials. Conversely, *Enterobacteriaceae* infections were associated with a high mortality rate (33%) despite 84% of patients having received appropriate antimicrobials (online Technical Appendix Table 14).

Multivariable logistic regression (Table 6) showed that third-generation cephalosporin resistance was associated with death (aOR 2.65, 95% CI 1.05–6.96; p = 0.042) and ICU admission (aOR 3.17, 95% CI 1.31–8.10; p =

Table 5. Logistic regression analysis of resistance trends for the gram-positive Global Antimicrobial Resistance Surveillance System pathogens *Staphylococcus aureus* and *Streptococcus pneumoniae* isolated from children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016*

Pathogen, resistance type, predictor variable	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p value	OR ratio (95% CI)	p value
S. aureus				
Methicillin				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	0.90 (0.18–4.93)	0.899	1.26 (0.23–7.59)	0.787
2011–2012	1.80 (0.47–8.90)	0.418	2.64 (0.62–14.48)	0.215
2013–2014	0.59 (0.10–3.42)	0.538	0.66 (0.10–4.19)	0.649
2015–2016	1.48 (0.35–7.61)	0.603	1.84 (0.39–10.47)	0.455
Patient age†				
Nonneonate	Ref	Ref	Ref	Ref
Neonate	0.18 (0.01–0.88)	0.094	0.14 (0.01–0.75)	0.064
Infection type‡				
Community-acquired	Ref	Ref	Ref	Ref
Hospital-acquired	6.21 (2.16–17.43)	<0.001	7.80 (2.51–24.81)	<0.001
S. pneumoniae§				
Penicillin				
Year of isolation				
2007–2008	Ref	Ref	Ref	Ref
2009–2010	0.60 (0.12–2.90)	0.525	0.70 (0.13–3.66)	0.669
2011–2012	0.52 (0.11–2.28)	0.385	0.42 (0.08–1.95)	0.269
2013–2014	0.72 (0.16–3.12)	0.663	0.77 (0.16–3.57)	0.737
2015–2016	1.87 (0.38–8.77)	0.424	1.89 (0.36–9.59)	0.436
Patient age, y				
≥5	Ref	Ref	Ref	Ref
<5	3.40 (1.63- 7.39)	0.001	3.87 (1.77–8.83)	<0.001

*OR, odds ratio; ref, referent.
†Ages are grouped into neonate (0–28 d) vs. nonneonate (≥29 d) or <5 y vs. ≥5 y, as appropriate for the organism.
‡Isolates were defined as hospital-acquired if taken >48 hours after patient admission.
§Analysis included community-acquired *Streptococcus pneumoniae* isolates only (n = 160).

0.013). Multivariable linear regression (online Technical Appendix Table 15) controlling for the same variables also showed an association between length of hospital stay among survivors and third-generation cephalosporin resistance (1.69-fold increase, 95% CI 1.21–2.37). Third-generation cephalosporin resistance was associated with a 2.26-fold increase in hospital costs among survivors (95% CI 1.51–3.36) (online Technical Appendix Tables 16, 17). According to this model, the median cost per admission would have been US $432.00 (IQR $333.30–$613.90) if all infections were third-generation cephalosporin sensitive and US $974.10 (IQR $751.60–$1,384.30) if all infections were third-generation cephalosporin resistant.

Discussion

In this hospitalized population of children in Cambodia, AMR levels were high, particularly among the gram-negative GLASS pathogens *K. pneumoniae*, *E. coli*, and *A. baumannii.* These organisms exhibited concerning resistance to WHO-recommended first-line sepsis treatment, emphasizing the urgent need for revised treatment guidelines (*4*). Few studies inform prevalence estimates of antimicrobial resistance in low- and middle-income countries in Asia, but compared with what is known, the high levels of gram-negative resistance reported here are not uncommon (*6*,*16*). For the gram-positive GLASS pathogens, *S. pneumoniae* resistance was broadly similar to that of the wider region (67.7% vs. 59.3% multidrug resistance, respectively) (*17*). Rates of methicillin-resistant *S. aureus* were comparatively lower; only 40.0% of hospital-acquired isolates were methicillin resistant compared with a regional average of 67.4% (*18*).

A major strength of this study is the observation of resistance trends over an extended period, something rarely possible in low- and middle-income countries because of lack of longstanding microbiology services. We found heterogeneous trends in resistance over time; resistance increased in some organisms (*Salmonella* Typhi) and decreased in others (*K. pneumoniae*). The most surprising temporal trend observed was a drop in the proportion of resistant *K. pneumoniae* isolates for most antimicrobials tested, in contrast to largely stable resistance levels in *E. coli*. For *K. pneumoniae* resistance by site of acquisition, in community-acquired isolates, resistance sharply declined in 2015–2016, perhaps suggesting loss of a plasmid coding for multiple resistance determinants. Confirming this trend will require a larger dataset from multiple sites in Cambodia and further analysis of the underlying resistance mechanisms at work using a method such as whole-genome sequencing. The genetic determinants of resistance in colonizing *K. pneumoniae* and *E. coli* isolates from

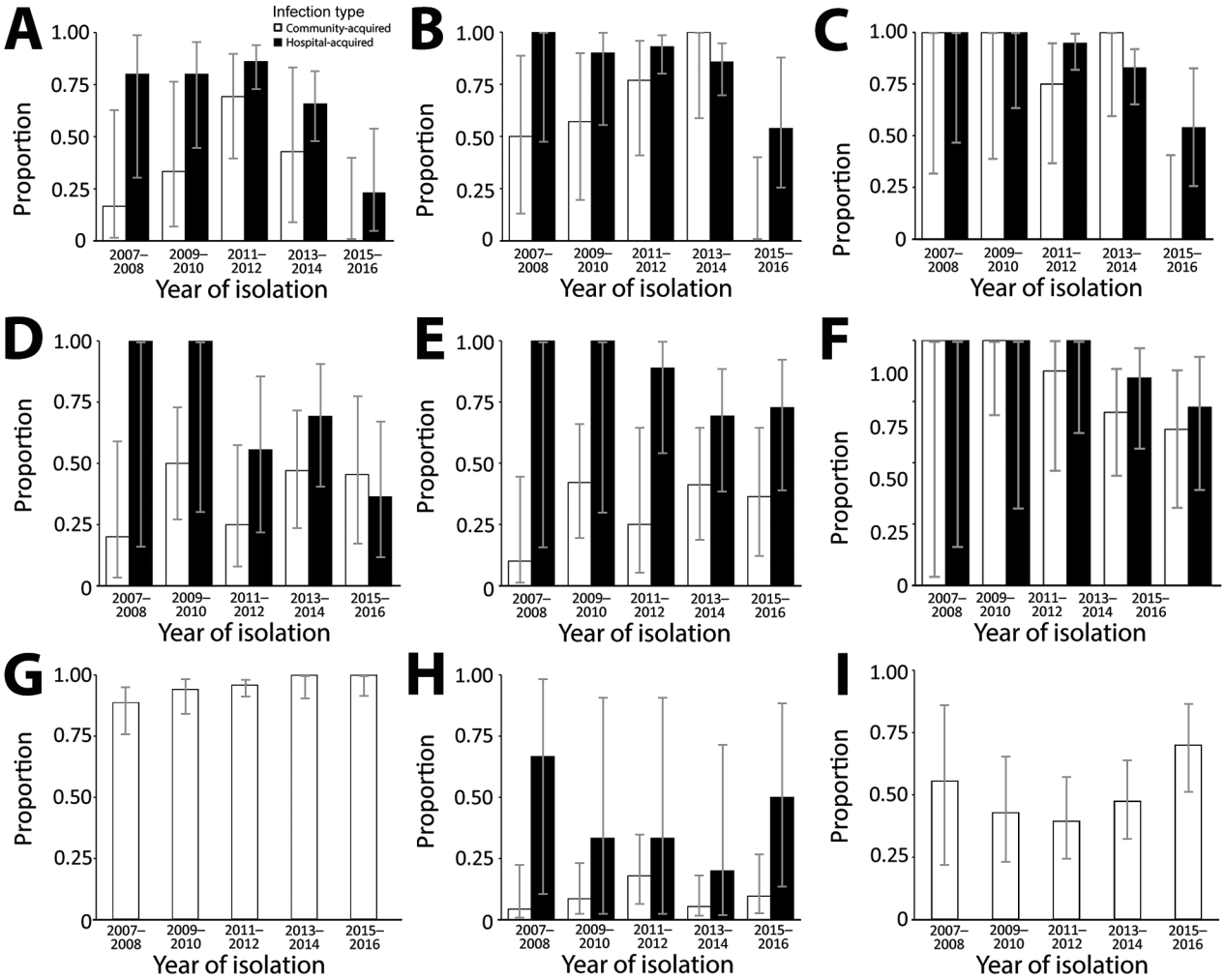

Figure 1. Antimicrobial resistance time trends, shown as proportion of resistant isolates from community-acquired and hospital-acquired infections, by year of isolation, in children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016. A) *Klebsiella pneumoniae* ampicillin–gentamicin resistance; B) *K. pneumoniae* third-generation cephalosporin resistance; C) *K. pneumoniae* multidrug resistance; D) *Escherichia coli* ampicillin–gentamicin resistance; E) *E. coli* third-generation cephalosporin resistance; F) *E. coli* multidrug resistance; G) *Salmonella enterica* serotype Typhi fluoroquinolone resistance; H) *Staphylococcus aureus* methicillin resistance; I) *Streptococcus pneumoniae* penicillin resistance. Isolates were defined as hospital-acquired if taken >48 hours after patient admission. Error bars indicate 95% CIs.

this population have been reported elsewhere (CE Moore, CM Parry, P Turner, NPJ Day, N Stoesser, unpub data; N Stoesser, C Turner, P Turner, BS Cooper, unpub data), whereas whole-genome sequencing of invasive isolates is ongoing. Loss of antimicrobial selective pressure leading to declining resistance may result from changes in national/regional antimicrobial supply or lack of active drug in antimicrobials used (*19*).

The number of hospital-acquired *K. pneumoniae* isolates peaked during 2011–2012. This peak may be the result of a genuine rise in the rate of hospital-acquired infections or the increased rate of blood culture sampling compared with previous years. From 2011–2012 onward, the proportion of resistant hospital-acquired *K. pneumoniae* isolates declined. This drop may be linked to maturation of a hospitalwide infection-control program implemented in 2010 (*20*) and enforced by prospective hospital-acquired infection surveillance from 2015 onward (*21*) or to the clinical microbiology service operating since 2012 with a strong focus on antimicrobial drug stewardship. Indeed, a recent study of prescribing practices at this hospital found 84%–89% of antimicrobial drug prescriptions were appropriate (*22*). The apparent success of these interventions suggests that they could be useful for combating AMR in similar settings. The perceived temporal drop in *K. pneumoniae* resistance could also be attributable to changing proportions of isolates from neonates over time; 46%–50% of isolates were from neonates in 2011–2014, dropping to 35% in 2015–2016.

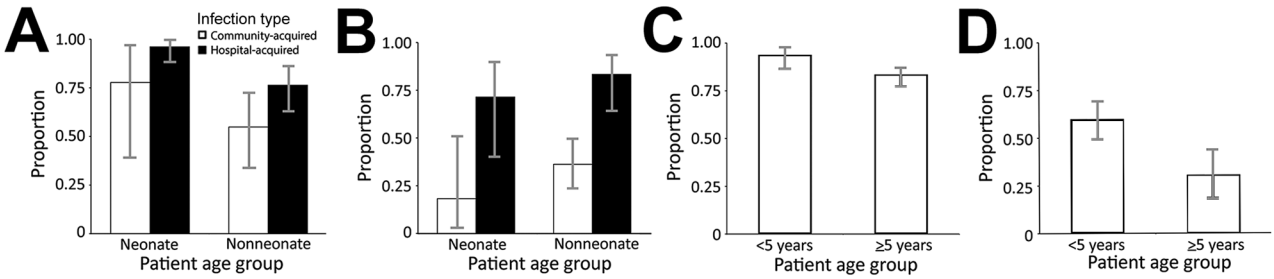

Figure 2. Antimicrobial resistance age trends, shown as proportion of resistant isolates from community-acquired and hospital-acquired infections, by patient age group, in children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016. A) *Klebsiella pneumoniae* third-generation cephalosporin resistance; B) *Escherichia coli* third-generation cephalosporin resistance; C) *Salmonella enterica* serotype Typhi multidrug resistance; D) *Streptococcus pneumoniae* penicillin resistance. Ages have been grouped into neonate (0–28 d) versus nonneonate (≥29 d) or <5 years versus ≥5 y, as appropriate for the organism. Isolates were defined as hospital-acquired if taken >48 hours after admission. Error bars indicate 95% CIs.

This study is unusual in that it directly compares different age groups of children, revealing AMR trends associated with age. Of note, the dominant pathogen in neonates, *K. pneumoniae*, was also more often resistant in neonates. For hospital-acquired isolates, this resistance may result from horizontal acquisition of resistant gram-negative organisms from hospital surfaces, as suggested by a recent multicenter study of sepsis in neonates (*2*). Indeed, colonization of neonates by resistant gram-negative organisms has been shown to be common at Angkor Hospital for Children and associated with subsequent invasive infection (*23*). For community-acquired isolates, vertical maternal transfer of resistant organisms may have a substantial role and is currently under investigation at this center.

Similarly, *Salmonella* Typhi from children <5 years of age was more often multidrug resistant than that from those ≥5 years of age. Isolates from younger children have greater genetic diversity (*24*), although how this diversity relates to increased AMR requires further investigation. In Cambodia, the most common indication for antimicrobial drug use is infections in children <5 years of age; thus, children in this age group may be exposed to more antimicrobial drugs, leading to greater resistance in organisms causing infection. That *S. pneumoniae* isolates were more often penicillin resistant in children <5 years of age is consistent with findings of previous work showing greater colonization of this age group by multidrug-resistant pneumococci (*25*). Vaccination may have a collateral benefit of reducing AMR (*26*), which suggests that it could be useful for combating *Salmonella* Typhi and *S. pneumoniae* resistance in low- and middle-income countries. Because 85% of *Salmonella* Typhi isolates are simultaneously fluoroquinolone resistant and multidrug resistant, few agents remain for treating typhoid in this population, placing even greater value on preventive measures such as vaccination. In January 2015, a 13-valent pneumococcal conjugate vaccine was introduced in Cambodia (*27*) with no catch-up campaign, meaning that only *S. pneumoniae* isolates from children born in or after December 2014 could have been affected, equating to 5 isolates in this dataset. Pneumococcal vaccination is thus unlikely to have had an appreciable effect on the AMR trends reported here.

The WHO Global Report on Surveillance identified a major gap in research comparing resource use in resistant versus nonresistant pathogens (*28*), an area that we addressed by demonstrating that resistance is associated with worse healthcare outcomes, including increased deaths and ICU admissions, delayed effective treatment, and more than doubled admission costs. Use of patient records allows these estimates to more closely reflect reality than modeled or ecologic analyses, although it is unclear whether this increased risk for adverse outcomes represents greater virulence, delayed treatment, or confounding. The observed outcome differences between *Enterobacteriaceae* and *A. baumannii* infections suggest either a true difference in virulence or that a proportion of *A. baumannii* isolates were contaminants, an uncertainty that highlights the difficulty of establishing the clinical significance of skin-colonizing organisms.

This study has several limitations. The data derive from a single nongovernmental hospital for children with limited numbers of isolates for some bacterial species; thus, trends and outcomes may not be representative of the wider region. The study was retrospective, and classification of community-acquired and hospital-acquired infections was limited by hospital database and clinical case note accuracy, meaning that some community-acquired infections may have actually been hospital-acquired infections. Widespread prehospitalization use of antimicrobials may have selected for resistant organisms (*29*). There were no restrictions to blood culture submission over time, but from early 2016 onward, clinicians were asked to focus on children requiring admission, which may have affected certain

Table 6. Multivariable logistic regression analysis of 129 hospital admission episodes for community-acquired monomicrobial gram-negative bacteremia in children at Angkor Hospital for Children, Siem Reap, Cambodia, 2007–2016*

	Death		ICU admission	
Predictor variable	OR (95% CI)	p value	OR (95% CI)	p value
Third-generation cephalosporin resistance	2.65 (1.05–6.96)	0.042	3.17 (1.31–8.10)	0.013
Neonate†	3.03 (1.14–8.31)	0.028	4.56 (1.83–12.16)	0.002
Male	0.81 (0.32–2.07)	0.659	0.81 (0.35–1.85)	0.616
Enterobacteriaceae‡	26.25 (4.43–511.1)	0.003	3.07 (1.05–9.67)	0.046
Malnourished§	2.11 (0.85–5.35)	0.111	2.19 (0.98–5.01)	0.059
Age <10 y	2.76 (0.40–56.29)	0.377	2.80 (0.60–20.70)	0.235

*Analysis used outcome (death or recovery) and ICU admission as the dependent variables. ICU, intensive care unit; OR, odds ratio.
†0–28 d of age.
‡*Acinetobacter baumannii* n = 29; *Enterobacteriaceae* n = 100 (consisting of *Escherichia coli*, n = 48; *Klebsiella pneumoniae,* n = 31; other pathogenic *Enterobacteriaceae* [consisting of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoea*, *Proteus*, and *Serratia* spp. n = 21]).
§Children <10 y of age only.

organisms (e.g., *Salmonella* Typhi). Microbiology practice variations over time meant that antimicrobial susceptibility testing was not consistent; however, we believe that the value of examining the evidence over a long period outweighed the effect that these variations may have had on results. For example, in 2009, the Clinical and Laboratory Standards Institute sensitivity zone size cutoffs for carbapenems and cephalosporins in *Enterobacteriaceae* increased, which could have resulted in a small number of isolates previously classed as sensitive being reclassified as resistant. The reported pre-2009 resistance levels are thus conservative and would not negate the downward resistance trends observed. In the outcome analysis, we did not consider prehospitalization factors, clinical diagnosis, and non-HIV/malnutrition co-occurring conditions because quantifying those could have introduced substantial reporting bias. Furthermore, our cost estimates may be higher than actual costs because we did not account for partial/shared doses and price fluctuations, suggesting that these cost estimates are most useful as a relative indication of cost burden.

In conclusion, the high rate of AMR in this setting of hospitalized children in Cambodia was associated with increased deaths and healthcare costs and threatens the effectiveness of first-line sepsis treatment. AMR represents a major threat to children's health globally (*5*,*6*), yet there is a dearth of data for children in low-resource settings (*3*–*6*). By reporting a decade of continuous AMR surveillance data, this study fills a gap in the understanding of antimicrobial drug resistance in children in Cambodia. In the context of the current global drive to combat AMR and the goal of the Fleming Fund to improve surveillance in low- and middle-income countries, our study demonstrates the feasibility and utility of undertaking accurate long-term antimicrobial drug resistance surveillance in these countries. The methods used here are reproducible in similar low-resource settings.

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Antimicrobial Resistance in Invasive Bacterial Infections in Hospitalized Children, Cambodia, 2007–2016

Technical Appendix

Methods

Classification of blood culture isolates

Coagulase-negative Staphylococci, *Micrococcus* species, and unspecified Gram-positive bacilli were classified as contaminants. Non-pathogenic environmental non-fermenting Gram-negatives were classified as of uncertain significance. All other bacterial species were considered as pathogens.

Management of Antimicrobial Susceptibility Testing data

Antimicrobial susceptibility testing (AST) results for BC isolates from 2007 to mid-2011 were interpreted as previously described (*1*). For BC isolates from mid-2011 onwards and CSFC isolates, disk-diffusion zone sizes and MIC results were available and re-interpreted using CLSI 2017 performance standards (*2*). Isolates classified as resistant, intermediate, or non-susceptible were analyzed as resistant. Changes in microbiology practice and guidelines meant species-specific AST panels varied over the study period. To account for this, pragmatic definitions of class resistance were used, and isolates only considered tested for multidrug resistant (MDR) status if they had undergone an appropriate minimum MDR test panel (Technical Appendix Table 1).

Technical Appendix Table 1. Definitions of antimicrobial resistance to be reported and minimum multidrug resistance (MDR) test panels for each organism/organism group*

Organism / Organism group	Resistance to be reported and definitions or resistance and sensitivity for antimicrobial classes	MDR test panel - minimum antimicrobial susceptibility tests required for isolate to be considered tested for MDR status
Salmonellae (*Salmonella* Typhi, *Salmonella* Paratyphi and Non-Typhoidal *Salmonellae*)	• Fluoroquinolone resistance = non-susceptibility to Ciprofloxacin (determined by Etest/MIC testing) and/or Nalidixic acid • Ceftriaxone resistance • Multidrug resistance = non-susceptibility to Ampicillin, Chloramphenicol and Co-trimoxazole	• Ampicillin • Chloramphenicol • Co-trimoxazole
Enterobacteriaceae (Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae)	• Ampicillin-Gentamicin resistance = non-susceptibility to both Ampicillin and Gentamicin • 3GC (Ceftazidime or Ceftriaxone or Cefotaxime or ESBL testing or Cefpodoxime testing, plus one of the above 3GC tests if Cefpodoxime resistant) • Carbapenem (Meropenem or Imipenem) • Multidrug resistance = non-susceptibility to ≥1 agent in ≥3 antimicrobial classes tested, excluding intrinsic resistance (as documented in CLSI guideline M100-S27)	• Ampicillin • Chloramphenicol • Ciprofloxacin • Co-trimoxazole • Gentamicin • 3GC o Ceftazidime or Ceftriaxone or Cefotaxime or ESBL testing or Cefpodoxime testing + one of the above 3GC tests if Cefpodoxime resistant • Carbapenem o Meropenem or Imipenem
Acinetobacter baumannii	• 3GC Ceftazidime or Ceftriaxone or Cefotaxime or ESBL testing or Cefpodoxime testing, plus one of the above 3GC tests if Cefpodoxime resistant) • Carbapenem (Meropenem or Imipenem) • Multidrug resistance (as for *Enterobacteriaceae*)	• Ciprofloxacin • Co-trimoxazole • Gentamicin • 3GC o Ceftazidime or Ceftriaxone or Cefotaxime or Cefpodoxime testing + one of the above 3GC tests if Cefpodoxime resistant • Carbapenem o Meropenem or Imipenem
Staphylococcus aureus	• Methicillin resistance = non-susceptibility to Cefoxitin or Oxacillin • Vancomycin resistance (as determined by Etest/MIC testing) • Multidrug resistance excluding Methicillin resistance (as for *Enterobacteriaceae*, excluding Methicillin resistant organisms, which by definition are also multidrug resistant)	• Cefoxitin • Ciprofloxacin • Co-trimoxazole • Erythromycin • Gentamicin • Penicillin
Streptococcus pneumoniae	• Oxacillin, plus Ceftriaxone Etest and Benzylpenicillin Etest if Oxacillin resistant • Macrolide/Lincosamide (Clindamycin or Erythromycin) • Multidrug resistance (as for *Enterobacteriaceae*)	• Chloramphenicol • Co-trimoxazole • Tetracycline • Macrolide/Lincosamide o Clindamycin or Erythromycin • Oxacillin + Ceftriaxone Etest and Benzylpenicillin Etest if Oxacillin resistant
Neisseria meningitidis	• Ceftriaxone resistance	Not applicable
Burkholderia pseudomallei	• Ceftazidime resistance as defined previously by Wuthiekanun et al (*3*) • Co-trimoxazole resistance as defined in CLSI guideline M45-A2 (*4*)	Not applicable
Haemophilus influenzae	• Ampicillin resistance • Ceftriaxone resistance • Multidrug resistance (as for *Enterobacteriaceae*)	• Ampicillin • Ceftriaxone • Chloramphenicol • Ciprofloxacin • Co-amoxiclav • Co-trimoxazole
Pseudomonas aeruginosa	• Ceftazidime resistance • Carbapenem (Meropenem or Imipenem) • Multidrug resistance (as for *Enterobacteriaceae*)	• Ceftazidime • Ciprofloxacin • Gentamicin • Carbapenem o Meropenem or Imipenem
Group A *Streptococcus*	• Macrolide/Lincosamide resistance (as for *S. pneumoniae*)	Not applicable

*MIC, minimum inhibitory concentration; Amp-Gent, ampicillin and gentamicin; 3GC, 3rd generation cephalosporin; ESBL, extended spectrum beta lactamase; Co-trimoxazole, trimethoprim-sulfamethoxazole.

Technical Appendix Table 2. Proportion of blood cultures taken by patient age group and year for 2012-2016 (n = 27,021)*

	Specimens per year (% of year total)					
Category	2012	2013	2014	2015	2016	Total (% of total)
Patient age group						
0-28 days†	453 (9.1)	573 (10.9)	739 (12.5)	851 (13.4)	955 (21.2)	3571 (13.2)
1-59 months	2,780 (55.6)	3,030 (57.6)	3,596 (61.0)	3,653 (57.5)	2,547 (56.4)	15,606 (57.8)
≥5 years	1,769 (35.4)	1,653 (31.4)	1,558 (26.4)	1,854 (29.2)	1,010 (22.4)	7,844 (29.0)
Specimen year total	5,002	5,256	5,893	6,358	4,512	27,021

*Accurate patient age data for all blood cultures taken (including negative cultures) was only routinely recorded from 2012 onwards, and thus proportions have only been provided for years 2012-2016.
†Neonate was defined as age 0-28 days.

Technical Appendix Table 3. Logistic regression analysis of *Salmonella* Typhi fluoroquinolone resistance trends (n = 322)*

	Univariable analysis		Multivariable analysis		
Predictor variable	OR (95% CI)	p value	OR (95% CI)	p value	AIC
Model 1 (year of isolation as a factor)					
Year of isolation (factor)					
2007-2008	-	-	-	-	113
2009-2010	2.05 (0.47-10.51)	0.345	1.85 (0.42-9.59)	0.422	
2011-2012	2.97 (0.82-10.37)	0.085	3.05 (0.83-10.74)	0.080	
2013-2014	4.03×10^{7} (6.26×10^{-45}-NA)	0.992	3.47×10^{7} (9.49×10^{-44}-NA)	0.992	
2015-2016	4.03×10^{7} (1.11×10^{-43}-NA)	0.992	4.14×10^{7} (6.26×10^{-43}-NA)	0.991	
Patient age, y					
≥5	-	-	-	-	
<5	4.48 (0.87-82.12)	0.151	4.57 (0.87-84.30)	0.150	
Model 2 (year of isolation as a continuous variable)					
Year of isolation (continuous)					
2007-2016	2.12 (1.28- 3.72)	0.005	2.14 (1.29-3.74)	0.005	109
Patient age, y					
≥5	-	-	-	-	
<5	4.48 (0.87-82.12)	0.151	4.71 (0.90-86.83)	0.141	

*OR, odds ratio; AIC, Akaike information criterion.

Technical Appendix Table 4. Resistance proportions for all standard antimicrobials tested by year of isolation for *Klebsiella pneumoniae*, *Escherichia coli* and *Streptococcus pneumoniae**

	n isolates	Year of isolation				
Pathogen, resistance type	n resistant/ n tested (%)	2007-2008	2009-2010	2011-2012	2013-2014	2015-2016
Klebsiella pneumoniae	146	11	17	56	42	20
Amp-Gent†	90/145 (62.1)	5/11 (45.5)	10/16 (62.5)	46/56 (82.1)	26/42 (61.9)	3/20 (15.0)
3GC	115/146 (78.8)	8/11 (72.7)	13/17 (76.5)	50/56 (89.3)	37/42 (88.1)	7/20 (35.0)
Carbapenem	1/142 (0.7)	0/8 (0.0)	0/16 (0.0)	0/56 (0.0)	1/42 (2.4)	0/20 (0.0)
MDR	108/132 (81.8)	8/8 (100.0)	12/12 (100.0)	45/50 (90.0)	36/42 (85.7)	7/20 (35.0)
Co-amoxiclav	101/146 (69.2)	8/11 (72.7)	11/17 (64.7)	47/56 (83.9)	30/42 (71.4)	5/20 (25.0)
Chloramphenicol	65/136 (47.8)	7/8 (87.5)	11/13 (84.6)	26/53 (49.1)	19/42 (45.2)	2/20 (10.0)
Ciprofloxacin	86/142 (60.6)	7/11 (63.6)	8/16 (50.0)	39/53 (73.6)	27/42 (64.3)	5/20 (25.0)
Co-trimoxazole	111/143 (77.6)	8/11 (72.7)	12/15 (80.0)	49/55 (89.1)	35/42 (83.3)	7/20 (35.0)
Escherichia coli	107	12	22	21	30	22
Amp-Gent	50/106 (47.2)	4/12 (30.8)	12/21 (57.1)	8/21 (38.1)	17/30 (56.7)	9/22 (40.9)
Ampicillin	101/107 (94.4)	10/12 (83.3)	21/22 (95.5)	21/21 (100.0)	28/30 (93.3)	21/22 (95.5)
Gentamicin	51/106 (48.1)	4/12 (33.3)	12/21 (57.1)	8/21 (38.1)	18/30 (60.0)	9/22 (40.9)
3GC	53/107 (49.5)	3/12 (25.0)	11/22 (50.0)	11/21 (52.4)	16/30 (53.3)	12/22 (54.5)
Carbapenem	0/98 (0.0)	0/3 (0.0)	0/22 (0.0)	0/21 (0.0)	0/30 (0.0)	0/22 (0.0)
MDR	69/84 (82.1)	3/3 (100.0)	13/13 (100.0)	15/16 (93.8)	23/30 (76.7)	15/22 (68.2)
Co-amoxiclav	57/107 (53.3)	7/12 (58.3)	13/22 (59.1)	14/21 (66.7)	12/30 (40.0)	11/22 (50.0)
Chloramphenicol	38/86 (44.2)	3/3 (100.0)	8/13 (61.5)	10/18 (55.6)	11/30 (36.7)	6/22 (27.3)
Ciprofloxacin	48/105 (45.7)	6/12 (50.0)	10/22 (45.5)	8/19 (42.1)	15/30 (50.0)	9/22 (40.9)
Co-trimoxazole	89/105 (84.8)	10/12 (83.3)	17/20 (85.0)	20/21 (95.2)	24/30 (80.0)	18/22 (81.8)
Streptococcus pneumoniae	166	17	36	40	41	32
Penicillin	73/144 (50.7)	5/9 (55.6)	10/23 (43.5)	16/39 (41.0)	20/41 (48.8)	22/32 (68.8)
Mac/Linc	49/165 (29.7)	5/17 (29.4)	10/35 (28.6)	12/40 (30.0)	11/41 (26.8)	11/32 (34.4)
MDR	63/93 (67.7)	0/0	0/0	10/20 (50.0)	26/41 (63.4)	27/32 (84.4)
Chloramphenicol	44/130 (33.8)	1/4 (25.0)	8/17 (47.1)	8/36 (22.2)	17/41 (41.5)	10/32 (31.3)
Co-trimoxazole	101/134 (75.4)	1/1 (100.0)	15/20 (75.0)	23/40 (57.5)	33/41 (80.5)	29/32 (90.6)
Tetracycline	87/93 (93.5)	0/0	0/0	18/20 (90.0)	39/41 (95.1)	30/32 (93.8)
Ceftriaxone	6/134 (4.5)	0/9 (0.0)	1/23 (4.3)	1/37 (2.7)	0/41 (0.0)	4/24 (16.7)

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. Amp-Gent, resistance of an isolate to both ampicillin and gentamicin; 3GC, 3rd generation cephalosporin; MDR, multidrug resistant; Co-amoxiclav, amoxicillin/clavulanic acid; Mac/Linc, resistance of an isolate to macrolides and/or lincosamides; Co-trimoxazole, trimethoprim-sulfamethoxazole.
†*Klebsiella pneumoniae* is intrinsically resistant to ampicillin, and thus ampicillin-gentamicin resistance in *K. pneumoniae* isolates is equivalent to gentamicin resistance.

Technical Appendix Table 5. Proportional numbers of *Klebsiella pneumoniae* isolates by patient age group and year of isolation (n = 146)

	Isolates per year of isolation (% of year group total)					
Category	2007-2008	2009-2010	2011-2012	2013-2014	2015-2016	Total (% of total)
Patient age group						
0-28 days*	1 (9.1)	5 (29.4)	26 (46.4)	21 (50.0)	7 (35.0)	60 (41.1)
1-59 months	7 (63.6)	10 (58.8)	24 (42.9)	17 (40.5)	10 (50.0)	68 (46.6)
≥5 years	3 (27.3)	2 (11.8)	6 (10.7)	4 (9.5)	3 (15.0)	18 (12.3)
Year group total	11	17	56	42	20	146

*Neonate was defined as age 0-28 days.

Technical Appendix Table 6. Mean disk diffusion zone of inhibition diameter size by year of isolation for *Klebsiella pneumoniae* and *Escherichia coli* isolates testing sensitive or resistant to Gentamicin, Ceftriaxone and Imipenem, 2012-2016*

Pathogen, resistance type	Year of isolation									
	2012		2013		2014		2015		2016	
	mean (SD)	n / tested	mean (SD)	n / tested	mean (SD)	n / tested	mean (SD)	n / tested	mean (SD)	n / tested
Klebsiella pneumoniae										
Gentamicin										
Sensitive	18.3 (1.0)	6/30	17.4 (1.8)	7/17	19.0 (3.7)	9/25	17.4 (1.3)	7/8	17.6 (1.1)	10/12
Resistant	7.7 (1.1)	24/30	8.1 (1.9)	10/17	8.1 (1.4)	16/25	10.0 (NA)	1/8	9.0 (1.4)	2/12
Ceftriaxone										
Sensitive	26.6 (1.3)	5/30	26.3 (1.2)	3/17	27.7 (2.5)	3/25	29.0 (2.8)	5/8	28.8 (1.8)	8/12
Resistant	6.8 (1.8)	25/30	8.0 (2.6)	14/17	9.4 (3.2)	22/25	7.0 (1.7)	3/8	9.0 (3.2)	4/12
Imipenem†										
Sensitive	27.8 (1.8)	30/30	27.5 (1.5)	17/17	26.9 (2.4)	24/25	27.9 (1.7)	8/8	27.1 (0.9)	7/7
Resistant		0/30		0/17	21.0 (NA)	1/25		0/8		0/7
Escherichia coli										
Gentamicin										
Sensitive	19.2 (4.3)	9/13	19.0 (1.4)	2/9	18.2 (1.5)	10/21	18.3 (1.6)	6/9	17.6 (1.1)	7/13
Resistant	7.3 (1.0)	4/13	7.0 (1.2)	7/9	7.7 (2.1)	11/21	6.7 (0.6)	3/9	6.7 (1.0)	6/13
Ceftriaxone										
Sensitive	26.2 (1.5)	5/13	29.2 (3.3)	5/9	27.7 (2.6)	9/21	29.8 (1.5)	6/9	29.3 (2.1)	4/13
Resistant	9.4 (4.3)	8/13	12.0 (6.5)	4/9	8.9 (4.0)	12/21	6.7 (1.2)	3/9	6.8 (1.2)	9/13
Imipenem†										
Sensitive	29.5 (2.4)	13/13	28.2 (2.0)	9/9	27.6 (2.4)	21/21	27.8 (1.6)	9/9	28.0 (1.1)	8/8
Resistant		0/13		0/9		0/21		0/9		0/8

*Zone of inhibition sizes were only available for complete years 2012-2016. SD, standard deviation.
†Note that at the end of 2016, carbapenem testing was switched from imipenem to meropenem. Thus 5 *Klebsiella pneumoniae* isolates and 5 *Escherichia coli* isolates from 2016 are not included in this table.

Technical Appendix Table 7. Resistance proportions by patient age group for Gram-negative Global Antimicrobial Resistance Surveillance System (GLASS) pathogens (n = 736)*

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Patient age group		
		0-28 days†	1-59 months	≥5 years
Klebsiella pneumoniae				
CAI	40 (27.4)	9	21	10
Amp-Gent	15/39 (38.5)	6/9 (66.7)	6/20 (30.0)	3/10 (30.0)
3GC	24/40 (60.0)	7/9 (77.8)	12/21 (57.1)	5/10 (50.0)
Carbapenem	0/36 (0.0)	0/9 (0.0)	0/18 (0.0)	0/9 (0.0)
MDR	23/33 (69.7)	7/9 (77.8)	11/15 (73.3)	5/9 (55.6)
HAI	106 (72.6)	51	47	8
Amp-Gent	75/106 (70.8)	44/51 (86.3)	27/47 (57.4)	4/8 (50.0)
3GC	91/106 (85.8)	49/51 (96.1)	37/47 (78.7)	5/8 (62.5)
Carbapenem	1/106 (0.9)	0/51 (0.0)	1/47 (2.1)	0/8 (0.0)
MDR	85/99 (85.9)	45/48 (93.8)	35/44 (79.5)	5/7 (71.4)
Escherichia coli				
CAI	69 (64.5)	11	50	8
Amp-Gent	27/68 (39.7)	2/11 (18.2)	20/49 (40.8)	5/8 (62.5)
3GC	23/69 (33.3)	2/11 (18.2)	15/50 (30.0)	6/8 (75.0)
Carbapenem	0/60 (0.0)	0/10 (0.0)	0/42 (0.0)	0/8 (0.0)
MDR	37/47 (78.7)	4/8 (0.5)	26/32 (81.3)	7/7 (100.0)
HAI	38 (35.5)	14	19	5
Amp-Gent	23/38 (60.5)	10/14 (71.4)	10/19 (52.6)	3/5 (60.0)
3GC	30/38 (78.9)	10/14 (71.4)	15/19 (78.9)	5/5 (100.0)
Carbapenem	0/38 (0.0)	0/14 (0.0)	0/19 (0.0)	0/5 (0.0)
MDR	32/37 (86.5)	12/14 (85.7)	15/18 (83.3)	5/5 (100.0)
Acinetobacter baumannii				
CAI	44 (58.7)	11	20	13
3GC	39/44 (88.6)	9/11 (81.8)	18/20 (90.0)	12/13 (92.3)
Carbapenem	4/44 (9.1)	2/11 (18.2)	2/20 (10.0)	0/13 (0.0)
MDR	8/43 (18.6)	2/10 (20.0)	4/20 (20.0)	2/13 (15.4)
HAI	31 (41.3)	5	21	5
3GC	31/31 (100.0)	5/5 (100.0)	21/21 (100.0)	5/5 (100.0)
Carbapenem	6/30 (20.0)	0/5 (0.0)	4/20 (20.0)	2/5 (40.0)
MDR	13/28 (46.4)	2/5 (40.0)	9/18 (50.0)	2/5 (40.0)
Salmonella Typhi				
Total (all CAI)	323	0	80	243
FQ	308/322 (95.7)		79/80 (98.8)	229/242 (94.6)
MDR	270/314 (86.0)		74/79 (93.7)	196/235 (83.4)

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Patient age group		
		0-28 days†	1-59 months	≥5 years
Salmonella Paratyphi A				
Total (all CAI)	44	0	3	41
FQ	10/44 (22.7)		1/3 (33.3)	9/41 (22.0)
MDR	0/43 (0.0)		0/3 (0.0)	0/40 (0.0)
Non-Typhoidal *Salmonellae*				
CAI	39 (95.1)	2	30	7
FQ	24/39 (61.5)	1/2 (50.0)	19/30 (63.3)	4/7 (57.1)
MDR	8/37 (21.6)	0/1 (0.0)	4/30 (13.3)	4/6 (66.7)
HAI	2 (4.9)	0	2	0
FQ	2/2 (100.0)		2/2 (100.0)	
MDR	1/2 (50.0)		1/2 (50.0)	

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. CAI, community-acquired infection; HAI, hospital-acquired infection; Amp-Gent, resistance of an isolate to both ampicillin and gentamicin; 3GC, 3rd generation cephalosporin; MDR, multidrug resistant; FQ, fluoroquinolone.
†Neonate was defined as age 0-28 days.

Technical Appendix Table 8. Resistance proportions by patient age group for Gram-positive Global Antimicrobial Resistance Surveillance System (GLASS) pathogens (n = 352)*

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Patient age group		
		0-28 days†	1-59 months	≥5 years
Staphylococcus aureus				
CAI	166 (89.2)	29	68	69
Methicillin	16/165 (9.7)	0/29 (0.0)	13/68 (19.1)	3/68 (4.4)
Vancomycin	0/5 (0.0)	0/0	0/2 (0.0)	0/3 (0.0)
MDR (excluding Methicillin resistance)	12/146 (8.2)	1/25 (4.0)	5/60 (8.3)	6/61 (9.8)
HAI	20 (10.8)	4	7	9
Methicillin	8/20 (40.0)	1/4 (25.0)	2/7 (28.6)	5/9 (55.6)
Vancomycin	0/4 (0.0)	0/1 (0.0)	0/2 (0.0)	0/1 (0.0)
MDR (excluding Methicillin resistance)	1/19 (5.3)	0/4 (0.0)	0/6 (0.0)	1/9 (11.1)
Streptococcus pneumoniae				
CAI	160 (96.4)	2	105	53
Penicillin	69/138 (50.0)	1/2 (50.0)	54/90 (60.0)	14/46 (30.4)
Mac/Linc	45/159 (28.3)	0/2 (0.0)	34/104 (32.7)	11/53 (20.8)
MDR	61/90 (67.8)	1/2 (50.0)	43/59 (72.9)	17/29 (58.6)
HAI	6 (3.6)	0	5	1
Penicillin	4/6 (66.7)		4/5 (80.0)	0/1 (0.0)
Mac/Linc	4/6 (66.7)		4/5 (80.0)	0/1 (0.0)
MDR	2/3 (66.7)		2/3 (66.7)	0/0

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. CAI, community-acquired infection; HAI, hospital-acquired infection; MDR, multidrug resistant; Mac/Linc, resistance of an isolate to macrolides and/or lincosamides.
†Neonate was defined as age 0-28 days.

Technical Appendix Table 9. Resistance proportions by patient age group for non-Global Antimicrobial Resistance Surveillance System (non-GLASS) pathogens (n = 253)*

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Patient age group		
		0-28 days†	1-59 months	≥5 years
Burkholderia pseudomallei				
Total (all CAI)	66	3	36	27
Ceftazidime	0/66 (0.0)	0/3 (0.0)	0/36 (0.0)	0/27 (0.0)
Co-trimoxazole	0/61 (0.0)	0/3 (0.0)	0/32 (0.0)	0/26 (0.0)
Haemophilus influenzae				
CAI	56 (98.2)	2	49	5
Ampicillin	30/55 (54.5)	1/2 (50.0)	27/48 (56.3)	2/5 (40.0)
Ceftriaxone	3/56 (5.4)	0/2 (0.0)	2/49 (4.1)	1/5 (20.0)
MDR	13/34 (38.2)	0/2 (0.0)	12/29 (41.4)	1/3 (33.3)
HAI	1 (1.8)	0	1	0
Ampicillin	0/1 (0.0)		0/1 (0.0)	
Ceftriaxone	0/1 (0.0)		0/1 (0.0)	
MDR	0/1 (0.0)		0/1 (0.0)	
Enterobacter cloacae				
CAI	12 (28.6)	3	6	3
Amp-Gent	6/12 (50.0)	2/3 (66.7)	3/6 (50.0)	1/3 (33.3)
3GC	8/12 (66.7)	3/3 (100.0)	4/6 (66.7)	1/3 (33.3)
Carbapenem	0/12 (0.0)	0/3 (0.0)	0/6 (0.0)	0/3 (0.0)
MDR	5/10 (50.0)	2/3 (66.7)	2/5 (40.0)	1/2 (50.0)
HAI	30 (73.2)	15	12	3
Amp-Gent	13/30 (43.3)	8/15 (53.3)	4/12 (33.3)	1/3 (33.3)
3GC	26/30 (86.7)	14/15 (93.3)	10/12 (83.3)	2/3 (66.7)
Carbapenem	3/29 (10.3)	1/15 (6.7)	2/11 (18.2)	0/3 (0.0)
MDR	13/27 (48.1)	8/15 (53.3)	4/9 (44.4)	1/3 (33.3)
Group A Streptococcus				
CAI	37 (97.4)	17	16	4
Mac/Linc	5/36 (13.9)	0/16 (0.0)	5/16 (31.3)	0/4 (0.0)
HAI	1 (2.6)	0	0	1
Mac/Linc	1/1 (100.0)			1/1 (100.0)
Pseudomonas aeruginosa				
CAI	20 (54.1)	1	13	6
Ceftazidime	2/17 (11.8)	0/1 (0.0)	1/11 (9.1)	1/5 (20.0)
Carbapenem	0/14 (0.0)	0/0	0/10 (0.0)	0/4 (0.0)
MDR	0/13 (0.0)	0/0	0/10 (0.0)	0/3 (0.0)
HAI	17 (45.9)	5	11	1
Ceftazidime	2/17 (11.8)	0/5 (0.0)	2/11 (18.2)	0/1 (0.0)
Carbapenem	2/16 (12.5)	0/5 (0.0)	2/10 (20.0)	0/1 (0.0)
MDR	0/16 (0.0)	0/5 (0.0)	0/10 (0.0)	0/1 (0.0)
Neisseria meningitidis				
Total (all CAI)	13	1	10	2
Ceftriaxone	1/13 (7.7)	0/1 (0.0)	1/10 (10.0)	0/2 (0.0)

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. CAI, community-acquired infection; HAI, hospital-acquired infection; Amp-Gent, resistance of an isolate to both ampicillin and gentamicin; 3GC, 3rd generation cephalosporin; MDR, multidrug resistant; Mac/Linc, resistance of an isolate to macrolides and/or lincosamides; Co-trimoxazole, trimethoprim-sulfamethoxazole.
†Neonate was defined as age 0-28 days.

Technical Appendix Table 10. Resistance proportions by year of isolation and site of acquisition for Gram-negative Global Antimicrobial Resistance Surveillance System (GLASS) pathogens (n = 736)*

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Year of isolation 2007-2008	2009-2010	2011-2012	2013-2014	2015-2016
Klebsiella pneumoniae						
CAI	40 (27.4)	6	7	13	7	7
Amp-Gent	15/39 (38.5)	1/6 (16.7)	2/6 (33.3)	9/13 (69.2)	3/7 (42.9)	0/7 (0.0)
3GC	24/40 (60.0)	3/6 (50.0)	4/7 (57.1)	10/13 (76.9)	7/7 (100.0)	0/7 (0.0)
Carbapenem	0/36 (0.0)	0/3 (0.0)	0/6 (0.0)	0/13 (0.0)	0/7 (0.0)	0/7 (0.0)
MDR	23/33 (69.7)	3/3 (100.0)	4/4 (100.0)	9/12 (75.0)	7/7 (100.0)	0/7 (0.0)
HAI	106 (72.6)	5	10	43	35	13
Amp-Gent	75/106 (70.8)	4/5 (80.0)	8/10 (80.0)	37/43 (86.0)	23/35 (65.7)	3/13 (23.1)
3GC	91/106 (85.8)	5/5 (100.0)	9/10 (90.0)	40/43 (93.0)	30/35 (85.7)	7/13 (53.8)
Carbapenem	1/106 (0.9)	0/5 (0.0)	0/10 (0.0)	0/43 (0.0)	1/35 (2.9)	0/13 (0.0)
MDR	85/99 (85.9)	5/5 (100.0)	8/8 (100.0)	36/38 (94.7)	29/35 (82.9)	7/13 (53.8)
Escherichia coli						
CAI	69 (64.5)	10	19	12	17	11
Amp-Gent	27/68 (39.7)	2/10 (20.0)	9/18 (50.0)	3/12 (25.0)	8/17 (47.1)	5/11 (45.5)
Ampicillin	64/69 (92.8)	8/10 (80.0)	18/19 (94.7)	12/12 (100.0)	16/17 (94.1)	10/11 (90.9)
Gentamicin	28/68 (41.2)	2/10 (20.0)	9/18 (50.0)	3/12 (25.0)	9/17 (52.9)	5/11 (45.5)
3GC	23/69 (33.3)	1/10 (10.0)	8/19 (42.1)	3/12 (25.0)	7/17 (41.2)	4/11 (36.4)
Carbapenem	0/60 (0.0)	0/1 (0.0)	0/19 (0.0)	0/12 (0.0)	0/17 (0.0)	0/11 (0.0)
MDR	37/47 (78.7)	1/1 (100.0)	10/10 (100.0)	7/8 (87.5)	12/17 (70.6)	7/11 (63.6)
HAI	38 (35.5)	2	3	9	13	11
Amp-Gent	23/38 (60.5)	2/2 (100.0)	3/3 (100.0)	5/9 (55.6)	9/13 (69.2)	4/11 (36.4)
Ampicillin	37/38 (97.4)	2/2 (100.0)	3/3 (100.0)	9/9 (100.0)	12/13 (92.3)	11/11 (100.0)
Gentamicin	23/38 (60.5)	2/2 (100.0)	3/3 (100.0)	5/9 (55.6)	9/13 (69.2)	4/11 (36.4)
3GC	30/38 (78.9)	2/2 (100.0)	3/3 (100.0)	8/9 (88.9)	9/13 (69.2)	8/11 (72.7)
Carbapenem	0/38 (0.0)	0/2 (0.0)	0/3 (0.0)	0/9 (0.0)	0/13 (0.0)	0/11 (0.0)
MDR	32/37 (86.5)	2/2 (100.0)	3/3 (100.0)	8/8 (100.0)	11/13 (84.6)	8/11 (72.7)
Acinetobacter baumannii						
CAI	44 (58.7)	2	4	17	16	5
3GC	39/44 (88.6)	2/2 (100.0)	3/4 (75.0)	14/17 (82.4)	16/16 (100.0)	4/5 (80.0)
Carbapenem	4/44 (9.1)	1/2 (50.0)	0/4 (0.0)	2/17 (11.8)	1/16 (6.3)	0/5 (0.0)
MDR	8/43 (18.6)	1/2 (50.0)	1/4 (25.0)	4/16 (25.0)	2/16 (12.5)	0/5 (0.0)
HAI	31 (41.3)	0	3	13	11	4
3GC	31/31 (100.0)		3/3 (100.0)	13/13 (100.0)	11/11 (100.0)	4/4 (100.0)
Carbapenem	6/30 (20.0)		1/2 (50.0)	3/13 (23.1)	2/11 (18.2)	0/4 (0.0)
MDR	13/28 (46.4)		1/2 (50.0)	5/11 (45.5)	6/11 (54.5)	1/4 (25.0)
Salmonella Typhi						
Total (all CAI)	323	44	51	146	40	42
FQ	308/322 (95.7)	39/44 (88.6)	48/51 (94.1)	139/145 (95.9)	40/40 (100.0)	42/42 (100.0)
Ceftriaxone	1/173 (0.6)	0/44 (0.0)	1/21 (4.8)	0/26 (0.0)	0/40 (0.0)	0/42 (0.0)
MDR	270/314 (86.0)	31/41 (75.6)	39/47 (83.0)	134/144 (93.1)	35/40 (87.5)	31/42 (73.8)
Salmonella Paratyphi A						
Total (all CAI)	44	3	0	0	35	6
FQ	10/44 (22.7)	3/3 (100.0)			4/35 (11.4)	3/6 (50.0)
Ceftriaxone	0/44 (0.0)	0/3 (0.0)			0/35 (0.0)	0/6 (0.0)
MDR	0/43 (0.0)	0/2 (0.0)			0/35 (0.0)	0/6 (0.0)
Non-Typhoidal *Salmonellae*						
CAI	39 (95.1)	7	4	7	9	12
FQ	24/39 (61.5)	4/7 (57.1)	2/4 (50.0)	4/7 (57.1)	6/9 (66.7)	8/12 (66.7)
Ceftriaxone	3/35 (8.6)	0/7 (0.0)	0/4 (0.0)	1/3 (33.3)	0/9 (0.0)	2/12 (16.7)
MDR	8/37 (21.6)	3/7 (42.9)	1/2 (50.0)	2/7 (28.6)	2/9 (22.2)	0/12 (0.0)
HAI	2 (4.9)	0	0	0	0	2
FQ	2/2 (100.0)					2/2 (100.0)
Ceftriaxone	0/2 (0.0)					0/2 (0.0)
MDR	1/2 (50.0)					1/2 (50.0)

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. CAI, community-acquired infection; HAI, hospital-acquired infection; Amp-Gent, resistance of an isolate to both ampicillin and gentamicin; 3GC, 3rd generation cephalosporin; MDR, multidrug resistant; FQ, fluoroquinolone.

†*Klebsiella pneumoniae* is intrinsically resistant to ampicillin, and thus ampicillin-gentamicin resistance in *K. pneumoniae* isolates is equivalent to gentamicin resistance.

Technical Appendix Table 11. Resistance proportions by year of isolation and site of acquisition for Gram-positive Global Antimicrobial Resistance Surveillance System (GLASS) pathogens (n = 352)*

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Year of isolation				
		2007-2008	2009-2010	2011-2012	2013-2014	2015-2016
Staphylococcus aureus						
CAI	166 (89.2)	23	35	40	37	31
Methicillin	16/165 (9.7)	1/23 (4.3)	3/35 (8.6)	7/39 (17.9)	2/37 (5.4)	3/31 (9.7)
Vancomycin	0/5 (0.0)	0/0	0/0	0/0	0/2 (0.0)	0/3 (0.0)
MDR (excluding Methicillin resistance)	12/146 (8.2)	7/22 (31.8)	3/27 (11.1)	1/29 (3.4)	0/37 (0.0)	1/31 (3.2)
HAI	20 (10.8)	3	3	3	5	6
Methicillin	8/20 (40.0)	2/3 (66.7)	1/3 (33.3)	1/3 (33.3)	1/5 (20.0)	3/6 (50.0)
Vancomycin	0/4 (0.0)	0/0	0/0	0/0	0/1 (0.0)	0/3 (0.0)
MDR (excluding Methicillin resistance)	1/19 (5.3)	1/3 (33.3)	0/3 (0.0)	0/2 (0.0)	0/5 (0.0)	0/6 (0.0)
Streptococcus pneumoniae						
CAI	160 (96.4)	17	34	39	40	30
Penicillin	69/138 (50.0)	5/9 (55.6)	9/21 (42.9)	15/38 (39.5)	19/40 (47.5)	21/30 (70.0)
Mac/Linc	45/159 (28.3)	5/17 (29.4)	9/33 (27.3)	11/39 (28.2)	10/40 (25.0)	10/30 (33.3)
MDR	61/90 (67.8)	0/0	0/0	10/20 (50.0)	25/40 (62.5)	26/30 (86.7)
HAI	6 (3.6)	0	2	1	1	2
Penicillin	4/6 (66.7)		1/2 (50.0)	1/1 (100.0)	1/1 (100.0)	1/2 (50.0)
Mac/Linc	4/6 (66.7)		1/2 (50.0)	1/1 (100.0)	1/1 (100.0)	1/2 (50.0)
MDR	2/3 (66.7)		0/0	0/0	1/1 (100.0)	1/2 (50.0)

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. CAI, community-acquired infection; HAI, hospital-acquired infection; MDR, multidrug resistant; Mac/Linc, resistance of an isolate to macrolides and/or lincosamides.

Technical Appendix Table 12. Resistance proportions by year of isolation and by site of acquisition for non-Global Antimicrobial Resistance Surveillance System (non-GLASS) pathogens (n = 253)*

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Year of isolation				
		2007-2008	2009-2010	2011-2012	2013-2014	2015-2016
Burkholderia pseudomallei						
Total (all CAI)	66	6	10	13	22	15
Ceftazidime	0/66 (0.0)	0/6 (0.0)	0/10 (0.0)	0/13 (0.0)	0/22 (0.0)	0/15 (0.0)
Co-trimoxazole	0/61 (0.0)	0/2 (0.0)	0/10 (0.0)	0/12 (0.0)	0/22 (0.0)	0/15 (0.0)
Haemophilus influenzae						
CAI	56 (98.2)	15	15	9	12	5
Ampicillin	30/55 (54.5)	5/14 (35.7)	10/15 (66.7)	7/9 (77.8)	8/12 (66.7)	0/5 (0.0)
Ceftriaxone	3/56 (5.4)	1/15 (6.7)	1/15 (6.7)	0/9 (0.0)	1/12 (8.3)	0/5 (0.0)
MDR	13/34 (38.2)	0/0	5/10 (50.0)	5/7 (71.4)	3/12 (25.0)	0/5 (0.0)
HAI	1 (1.8)	0	0	0	0	1
Ampicillin	0/1 (0.0)					0/1 (0.0)
Ceftriaxone	0/1 (0.0)					0/1 (0.0)
MDR	0/1 (0.0)					0/1 (0.0)
Enterobacter cloacae						
CAI	12 (28.6)	1	4	3	3	1
Amp-Gent	6/12 (50.0)	1/1 (100.0)	3/4 (75.0)	2/3 (66.7)	0/3 (0.0)	0/1 (0.0)
3GC	8/12 (66.7)	1/1 (100.0)	3/4 (75.0)	3/3 (100.0)	1/3 (33.3)	0/1 (0.0)
Carbapenem	0/12 (0.0)	0/1 (0.0)	0/4 (0.0)	0/3 (0.0)	0/3 (0.0)	0/1 (0.0)
MDR	5/10 (50.0)	1/1 (100.0)	2/2 (100.0)	2/3 (66.7)	0/3 (0.0)	0/1 (0.0)
HAI	30 (73.2)	1	2	5	14	8
Amp-Gent	13/30 (43.3)	0/1 (0.0)	2/2 (100.0)	3/5 (60.0)	6/14 (42.9)	2/8 (25.0)
3GC	26/30 (86.7)	0/1 (0.0)	2/2 (100.0)	4/5 (80.0)	13/14 (92.9)	7/8 (87.5)
Carbapenem	3/29 (10.3)	0/0	0/2 (0.0)	0/5 (0.0)	2/14 (14.3)	1/8 (12.5)
MDR	13/27 (48.1)	0/0	0/0	3/5 (60.0)	7/14 (50.0)	3/8 (37.5)
Group A Streptococcus						
CAI	37 (97.4)	2	6	6	13	10
Mac/Linc	5/36 (13.9)	0/2 (0.0)	1/5 (20.0)	0/6 (0.0)	2/13 (15.4)	2/10 (20.0)
HAI	1 (2.6)	0	0	0	0	1
Mac/Linc	1/1 (100.0)					1/1 (100.0)
Pseudomonas aeruginosa						
CAI	20 (54.1)	6	5	2	3	4
Ceftazidime	2/17 (11.8)	0/3 (0.0)	1/5 (20.0)	1/2 (50.0)	0/3 (0.0)	0/4 (0.0)
Carbapenem	0/14 (0.0)	0/1 (0.0)	0/4 (0.0)	0/2 (0.0)	0/3 (0.0)	0/4 (0.0)

Pathogen, site of acquisition, resistance type	n isolates n resistant/n tested (%)	Year of isolation				
		2007-2008	2009-2010	2011-2012	2013-2014	2015-2016
MDR	0/13 (0.0)	0/0	0/4 (0.0)	0/2 (0.0)	0/3 (0.0)	0/4 (0.0)
HAI	17 (45.9)	1	1	5	6	4
Ceftazidime	2/17 (11.8)	0/1 (0.0)	0/1 (0.0)	0/5 (0.0)	2/6 (33.3)	0/4 (0.0)
Carbapenem	2/16 (12.5)	0/0	0/1 (0.0)	1/5 (20.0)	0/6 (0.0)	1/4 (25.0)
MDR	0/16 (0.0)	0/0	0/1 (0.0)	0/5 (0.0)	0/6 (0.0)	0/4 (0.0)
Neisseria meningitidis						
Total (all CAI)	13	6	3	0	2	2
Ceftriaxone	1/13 (7.7)	0/6 (0.0)	1/3 (33.3)		0/2 (0.0)	0/2 (0.0)

*Resistance proportions have been reported as number of resistant isolates out of actual number of isolates tested. CAI, community-acquired infection; HAI, hospital-acquired infection; Amp-Gent, resistance of an isolate to both ampicillin and gentamicin; 3GC, 3rd generation cephalosporin; MDR, multidrug resistant; Mac/Linc, resistance of an isolate to macrolides and/or lincosamides; Co-trimoxazole, trimethoprim-sulfamethoxazole.

Technical Appendix Table 13. Univariable logistic regression analysis of demographic characteristics, clinical characteristics and outcomes, compared by resistance and by outcome, for admission episodes due to community-acquired monomicrobial Gram-negative bacteremia (n = 129)*

Category	Overall	3GC Resistance			Outcome		
		Sensitive (n = 66)	**Resistant (n = 63)**	**p value**	**Survived (n = 95)**	**Died (n = 34)**	**p value**
Demographic characteristics							
Median age in months (IQR)	8.6 (0.8-29.2)	4.5 (0.4-20.7)	11.9 (1.2-63.0)	0.037	10.2 (1.5-42.6)	1.4 (0.1-11.7)	0.002
Neonate (%)†	34 (26%)	19 (29%)	15 (24%)	0.659	19 (20%)	15 (44%)	0.012
Male (%)	72 (56%)	38 (58%)	34 (54%)	0.814	51 (54%)	21 (62%)	0.540
Clinical characteristics							
Malnourished (%)‡	53 (41%)	28 (42%)	25 (40%)	0.891	33 (35%)	20 (59%)	0.025
***Enterobacteriaceae* infection§ (%)**	100 (78%)	63 (96%)	37 (59%)	<0.001	67 (71%)	33 (97%)	0.003
HIV positive (%)	1 (1%)	0 (0%)	1 (2%)	-	0 (0%)	1 (3%)	-
3GC resistance (%)	63 (49%)	-	-	-	46 (48%)	17 (50%)	1
Outcomes							
Death (%)	34 (26%)	17 (26%)	17 (27%)	1	-	-	-
ICU admission (%)	56 (43%)	25 (38%)	31 (49%)	0.263	26 (27%)	30 (88%)	<0.001
Appropriate treatment received (%)¶	98 (76%)	62 (94%)	36 (57%)	<0.001	74 (78%)	24 (71%)	0.534
Median days to treatment (IQR)#	0 (0-1.0)	-	-	-	0 (0-2)	0 (0 – 0)	0.087
Survived	0 (0-2.0)	0 (0-0)	2 (0.3-3.8)	<0.001	-	-	-
Died	0 (0-0)	0 (0-0)	0.5 (0-1.8)	0.004	-	-	-
Median length of stay (IQR)**	7.0 (4.0-10.0)	-	-	-	8.0 (6.0-12.5)	3.0 (2.0-4.8)	<0.001
Survived	8.0 (6.0-12.5)	8.0 (5.0-11.0)	9.0 (6.3-18.0)	0.179	-	-	-
Died	3.0 (2.0-4.8)	2.0 (1.0-4.0)	4.0 (2.0-7.0)	0.030	-	-	-

***Percentages are shown as a percentage of the column total. *P*-values were calculated using the chi-squared test with Yates correction for categorical variables, and the Mann-Whitney-Wilcoxon test for continuous variables. 3GC, 3rd generation cephalosporin; IQR, interquartile range; HIV, human immunodeficiency virus; ICU, intensive care unit.**

†Neonate was defined as age 0-28 days and non-neonate as age ≥29 days.

‡Malnutrition in children aged under ten years was defined as per WHO AnthroPlus software (*5*). Lack of height measurements meant it was not possible to classify malnutrition in children aged over ten years.

§*Acinetobacter baumannii* n = 29, *Enterobacteriaceae* n = 100 (consisting of *Escherichia coli* n = 48, *Klebsiella pneumoniae* n = 31, other pathogenic ***Enterobacteriaceae* consisting of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoea*, *Proteus*, and *Serratia* species n = 21).**

¶Appropriate treatment was defined as receipt of an antimicrobial to which the organism was susceptible.

#Time to appropriate treatment was defined as days between day of admission and day of receipt.

**Length of stay was calculated separately for discharged patients and patients who died, taking day of admission as day one.

Technical Appendix Table 14. Comparison of outcomes between *Enterobacteriaceae* infections and *Acinetobacter baumannii* infections in admission episodes due to community-acquired monomicrobial Gram-negative bacteremia (n = 129)*

Outcome	***Enterobacteriaceae* (n=100)**	***Acinetobacter baumannii* (n=29)**
3GC resistance	37 (37%)	26 (90%)
Death (%)	33 (33%)	1 (3%)
ICU admission (%)	46 (46%)	10 (35%)
Appropriate treatment received (%)†	84 (84%)	14 (48%)
Median days to treatment (IQR)‡		
Survived	0 (0-1.0)	0 (0-4)
Died	0 (0-0)	0 (0-0)
Median length of stay (IQR)§		
Survived	9.0 (7.0-15.0)	7.0 (4.8-10.3)
Died	3.0 (2.0-5.0)	3.0 (3.0-3.0)

*Percentages are shown as a percentage of the column total. 3GC, 3rd generation cephalosporin; ICU, intensive care unit; IQR, interquartile range.
†Appropriate treatment was defined as receipt of an antimicrobial to which the organism was susceptible.
‡Time to appropriate treatment was defined as days between day of admission and day of receipt.
§Length of stay was calculated separately for discharged patients and patients who died, taking day of admission as day one.

Technical Appendix Table 15. Length of stay in survivors: linear regression analysis of admission episodes due to community-acquired monomicrobial Gram-negative bacteremia, taking length of stay in survivors as the dependent variable (n = 129)*

Predictor variable	**Log coefficients (95% CI)**	**Coefficients (95% CI)**	**p value**
3GC resistance	0.52 (0.19-0.86)	1.69 (1.21-2.37)	0.003
Neonate†	0.54 (0.17-0.91)	1.72 (1.19-2.49)	0.005
Male	-0.17 (-0.48-0.14)	0.84 (0.62-1.15)	0.280
Enterobacteriaceae infection‡	0.52 (0.14-0.89)	1.68 (1.15-2.44)	0.008
Malnourished§	0.34 (0.03-0.65)	1.40 (1.03-1.92)	0.032
Age under 10 years	-0.008 (-0.49-0.47)	0.99 (0.62-1.60)	0.972

*The first column shows the coefficients from the log-transformed model, while the second column shows the coefficients that have been back-transformed and are now on a multiplicative scale. 3GC, 3rd generation cephalosporin.
†Neonate was defined as age 0-28 days and non-neonate as age ≥29 days.
‡*Acinetobacter baumannii* n = 29, *Enterobacteriaceae* n = 100 (consisting of *Escherichia coli* n = 48, *Klebsiella pneumoniae* n = 31, other pathogenic *Enterobacteriaceae* consisting of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoea*, *Proteus*, and *Serratia* species n = 21).
§Malnutrition in children aged under ten years was defined as per WHO AnthroPlus software (*5*). Lack of height measurements meant it was not possible to classify malnutrition in children aged over ten years.

Technical Appendix Table 16. Total admission cost in survivors: Linear regression analysis of admission episodes due to community-acquired monomicrobial Gram-negative bacteremia, taking total admission cost in survivors as the dependent variable (n = 129)*

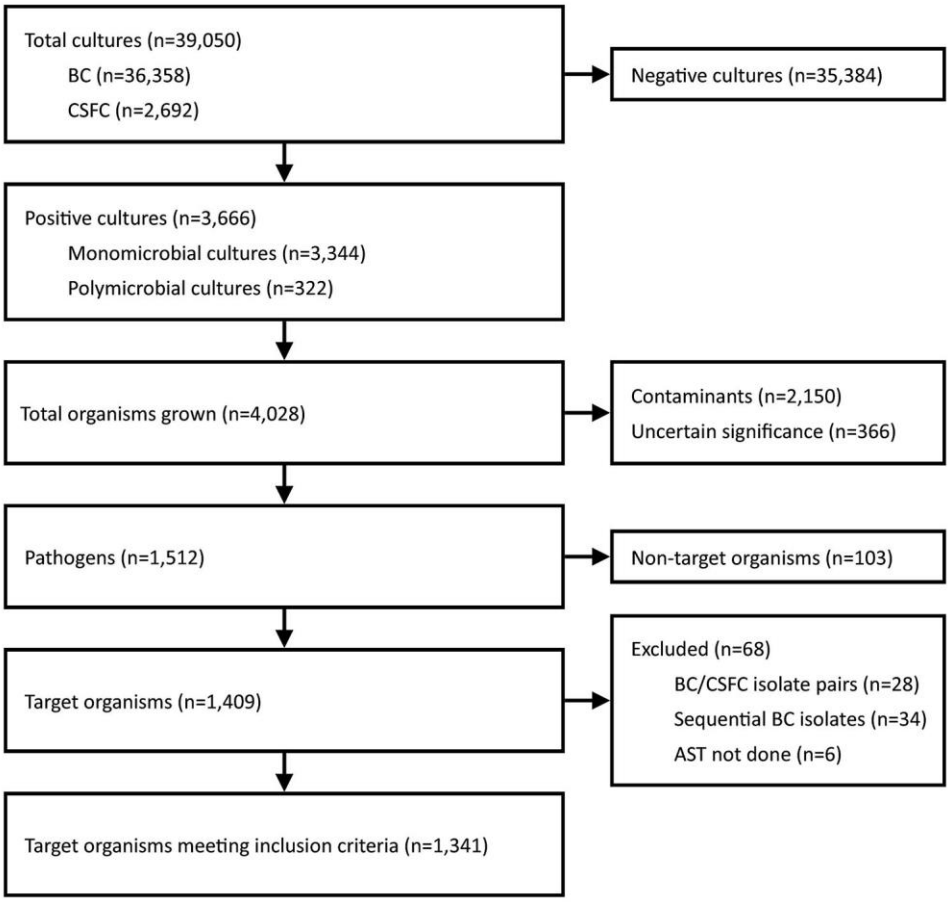
Predictor variable	**Log coefficient (95% CI)**	**Coefficient (95% CI)**	**p value**
3GC resistance	0.81 (0.42-1.21)	2.26 (1.51-3.36)	<0.001
Neonate†	1.16 (0.72-1.60)	3.20 (2.06-4.96)	<0.001
Male	-0.26 (-0.63-0.11)	0.77 (0.54-1.11)	0.164
Enterobacteriaceae infection‡	0.81 (0.37-1.25)	2.25 (1.44-3.51)	0.001
Malnourished§	0.37 (0.007-0.74)	1.45 (1.01-2.10)	0.046
Age under 10 years	-0.06 (-0.63-0.51)	0.94 (0.54-1.66)	0.835

*Total cost of admission defined as the sum of the cost of stay and cost of antimicrobials. The first column shows coefficients from the log-transformed model, while the second column shows coefficients that have been back-transformed, which are now on a multiplicative scale. 3GC, 3rd generation cephalosporin.
†Neonate was defined as age 0-28 days and non-neonate as age ≥29 days.
‡*Acinetobacter baumannii* n = 29, *Enterobacteriaceae* n = 100 (consisting of *Escherichia coli* n = 48, *Klebsiella pneumoniae* n = 31, other pathogenic *Enterobacteriaceae* consisting of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoea*, *Proteus*, and *Serratia* species n = 21).
§Malnutrition in children aged under ten years was defined as per WHO AnthroPlus software (*5*). Lack of height measurements meant it was not possible to classify malnutrition in children aged over ten years.

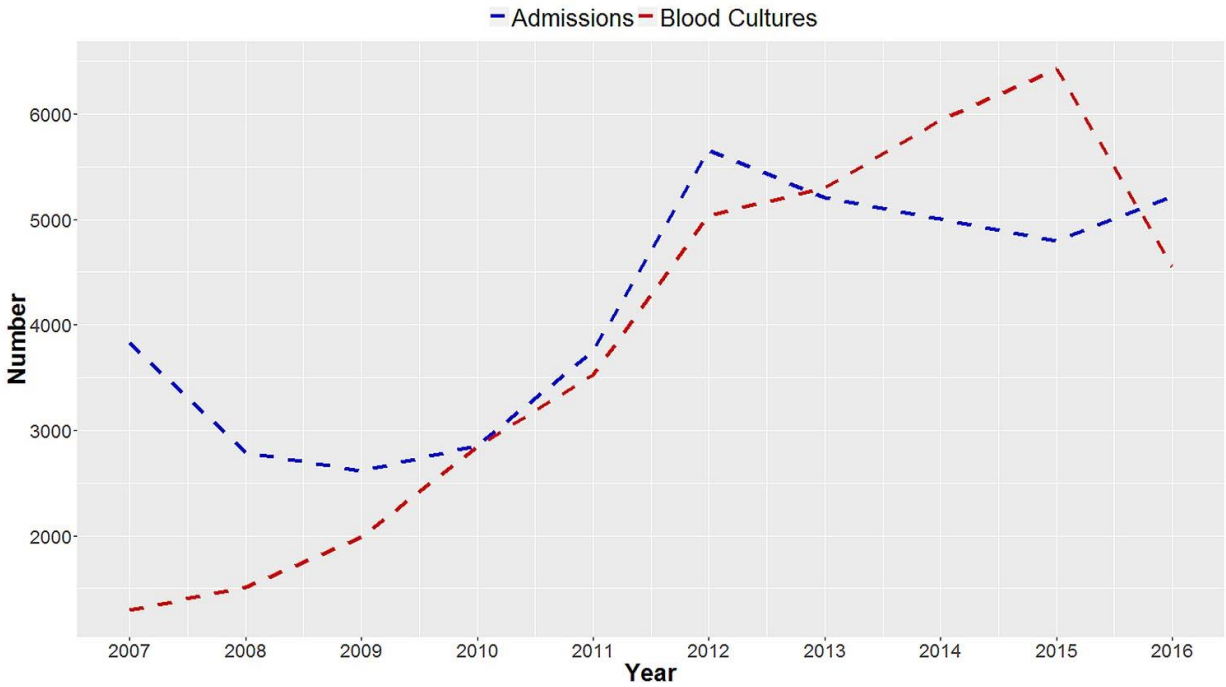
Technical Appendix Table 17. Median costs of admission episodes due to community-acquired monomicrobial Gram-negative bacteremia (n = 129)*

Cost	**Total**	**3GC sensitive**	**3GC resistant**
Antibiotic costs	11.31 (5.97-91.30)	8.80 (5.88-18.08)	14.85 (6.67-574.90)
Cost of stay	500.00 (350.00-1010.00)	450.00 (350.00-909.00)	500.00 (350.00-1246.00)
Total cost	515.4 (354.80-1247.00)	458.0 (355.30-915.00)	804.8 (354.60-1831.00)

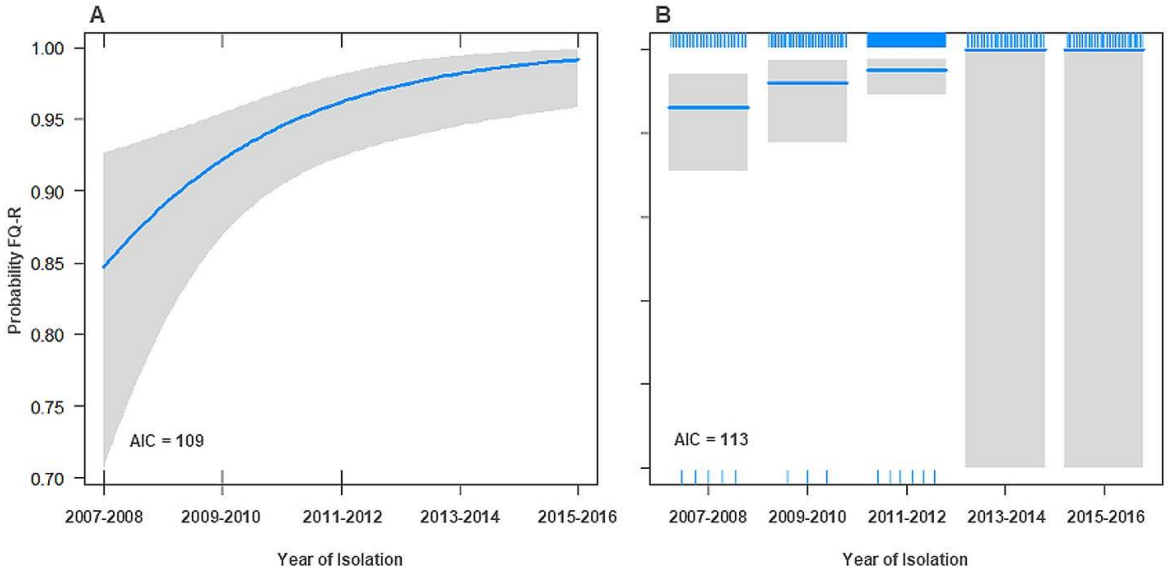
**Acinetobacter baumannii* n = 29, *Enterobacteriaceae* n = 100 (consisting of *Escherichia coli* n = 48, *Klebsiella pneumoniae* n = 31, other pathogenic *Enterobacteriaceae* consisting of *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoea*, *Proteus*, and *Serratia* species n = 21).
Costs are listed as median cost (interquartile range). The cost of stay excluded costs of all drugs (antimicrobials and non-antimicrobials). All costs are in 2017 US $. Costs are only reported for the cases that recovered so that death does not confer a cost advantage. 3GC, 3rd generation cephalosporin.



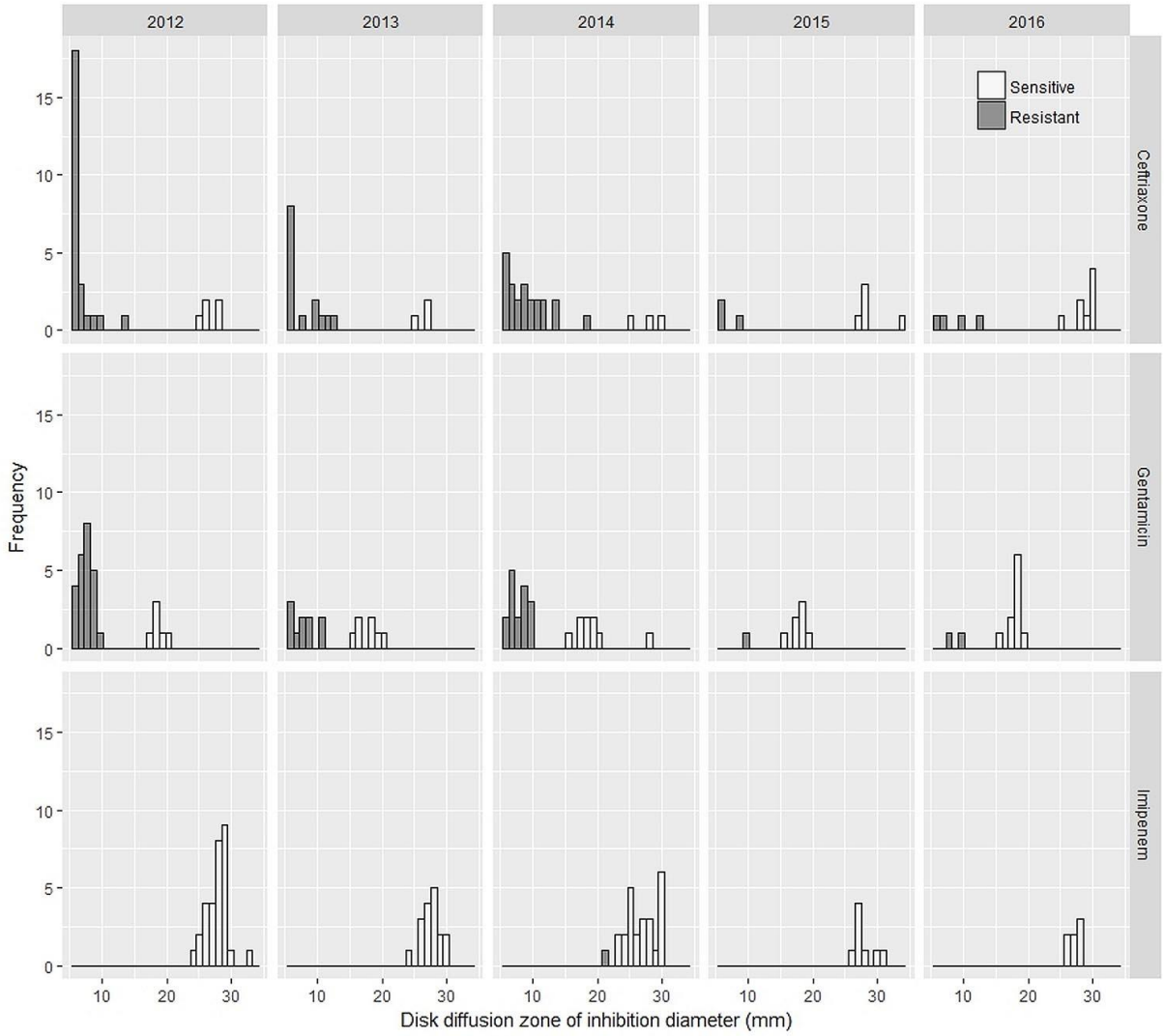
Technical Appendix Figure 1. Study profile. BC, blood culture; CSFC, cerebrospinal fluid culture; AST, antimicrobial susceptibility testing.



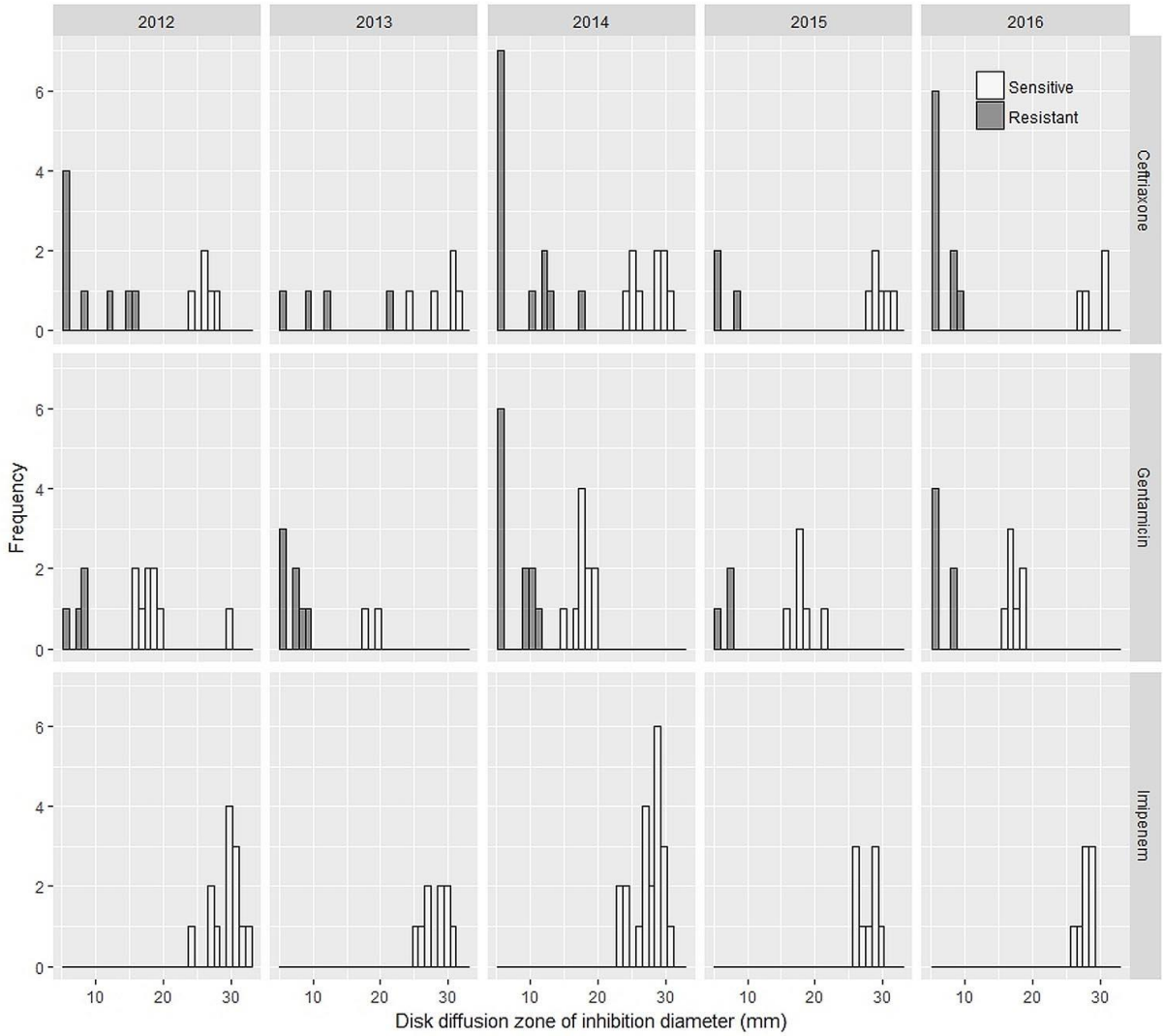
Technical Appendix Figure 2. Inpatient department admissions and blood cultures sent over time.



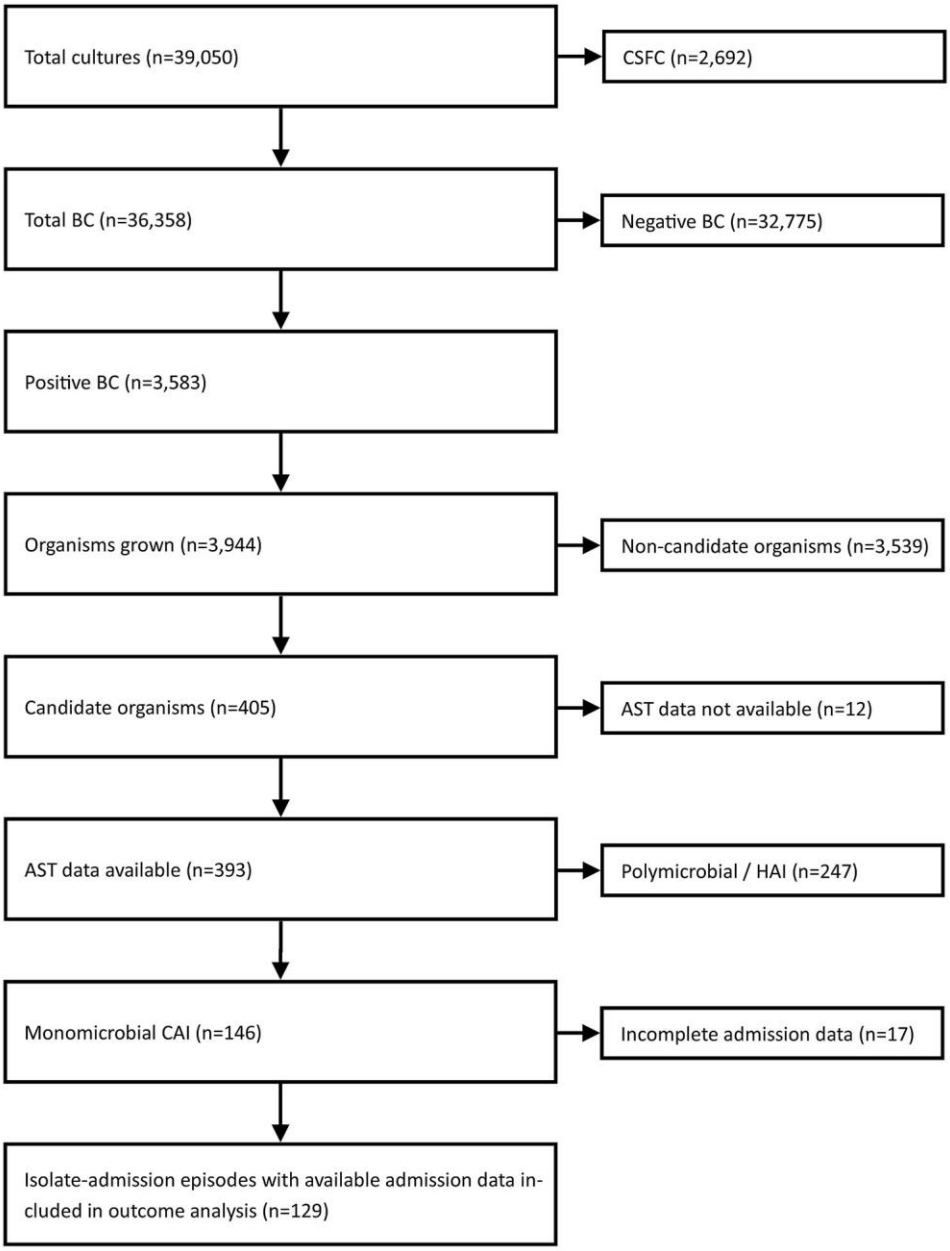
Technical Appendix Figure 3. Predicted probability of fluoroquinolone resistant *Salmonella* Typhi isolates by Year of Isolation, from multivariable logistic regression models with time modelled as a continuous variable (A) or a factor (B). FQ-R, fluoroquinolone resistance; AIC, Akaike Information Criterion.



Technical Appendix Figure 4. Histogram of disk diffusion zone of inhibition diameter sizes by year for *Klebsiella pneumoniae* isolates testing sensitive or resistant to Gentamicin, Ceftriaxone and Imipenem, 2012-2016.



Technical Appendix Figure 5. Histogram of disk diffusion zone of inhibition diameter sizes by year for *Escherichia coli* isolates testing sensitive or resistant to Gentamicin, Ceftriaxone and Imipenem, 2012-2016.



Technical Appendix Figure 6. Profile of isolate-admission episodes included in outcome analysis. BC, blood culture; CSFC, cerebrospinal fluid culture; AST, antimicrobial susceptibility testing; HAI, hospital-acquired infection; CAI, community-acquired infection.

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