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14-year trends and resistance patterns of blood and cerebrospinal fluid cultures in children under three years old

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Abstract:	Objectives Bacterial infections are a major cause of UK paediatric hospitalisations, yet longitudinal data on causative organisms or antimicrobial resistance are scarce. This retrospective analysis describes trends in blood and cerebrospinal fluid (CSF) cultures and resistance patterns in children under three years old from a large UK centre. Methods All culture results, and resistance data for Gram-negative rods (GNR) in blood cultures, collected between January 2005 and December 2018 were extracted from Oxford University Hospitals NHS Foundation Trust microbiology database. Results Of 49,298 samples, 6.7% of blood and 3.1% of CSF cultures were positive for bacterial growth; 2.3% and 1.1% respectively grew pathogens. Number of cultures taken increased over time; the proportion growing pathogens declined. Resistance of GNR to first-line antimicrobials was 9.3% to gentamicin (neonatal units), and 17.1% and 25.8% to ceftriaxone (paediatric ED and wards respectively). Resistance to any two of ceftriaxone, ciprofloxacin, gentamicin, or meropenem was ≤6% in both areas. Conclusions The proportion of positive cultures declined over time. Resistance of GNR to empirical antimicrobials were observed, but resistance to a second agent were lower. Our study informs clinician decisions on when, and to which antimicrobials, to escalate if a child is not improving on empirical therapy.
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14-year trends and resistance patterns of blood and cerebrospinal fluid cultures in children under three years old

Running title: 14-year trends and resistance in children under three

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Keywords

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Abstract

Objectives

Bacterial infections are a major cause of UK paediatric hospitalisations, yet longitudinal data on causative organisms or antimicrobial resistance are scarce. This retrospective analysis describes trends in blood and cerebrospinal fluid (CSF) cultures and resistance patterns in children under three years old from a large UK centre.

Methods

All culture results, and resistance data for Gram-negative rods (GNR) in blood cultures, collected between January 2005 and December 2018 were extracted from Oxford University Hospitals NHS Foundation Trust microbiology database.

Results

Of 49,298 samples, 6.7% of blood and 3.1% of CSF cultures were positive for bacterial growth; 2.3% and 1.1% respectively grew pathogens. Number of cultures taken increased over time; the proportion growing pathogens declined. Resistance of GNR to first-line antimicrobials was 9.3% to gentamicin (neonatal units), and 17.1% and 25.8% to ceftriaxone (paediatric ED and wards respectively). Resistance to any two of ceftriaxone, ciprofloxacin, gentamicin, or meropenem was ≤6% in both areas.

Conclusions

The proportion of positive cultures declined over time. Resistance of GNR to empirical antimicrobials were observed, but resistance to a second agent were lower. Our study informs clinician decisions on when, and to which antimicrobials, to escalate if a child is not improving on empirical therapy.

Highlights

- Number of cultures taken increased over time, but proportion positive declined
- Most blood cultures were taken from children under 2 days old but few were positive
- Cases of vaccine-preventable organisms were few and declined over time
- Resistance to empirical agents exist but resistance to a second agent were low

Introduction

Bacterial sepsis is a leading cause of morbidity and mortality in young infants accounting for approximately 6% of neonatal deaths worldwide, but its management is threatened by rising antimicrobial resistance (AMR). [1] [2] Resistance in Gram-negative bacteria is particularly concerning, with increasing multi-drug resistance and evidence that poor empirical therapy choice can result in poor prognosis. [3] [4] Improved surveillance is highlighted as one of the key recommendations to counter AMR in the World Health Organization's global action plan on antimicrobial resistance, and also in the 5-year antimicrobial resistance strategy of the United Kingdom (UK) Department of Health. [5] [6]

While such data exist in adults, there is currently limited information from the paediatric population. The Antimicrobial Resistance and Prescribing in European Children (ARPEC) project provides a validated cross-sectional surveillance tool to evaluate hospital antimicrobial prescribing patterns. [7] Longitudinal data on causative organisms and resistance, however, still remains limited and are often focused on specific organisms or subpopulations. Further, with marked regional variation in both organism detection and resistance patterns, it is important to tailor any AMR interventions to local data and prescribing guidelines, a point that was also highlighted in the UK 5-year strategy. [6] Paediatricians currently lack robust data to help guide decisions on antimicrobial choice, especially in the first few hours before the full identity of the organism or detailed sensitivity results may be known.

We, therefore, conducted a retrospective study of blood and cerebrospinal fluid (CSF) cultures taken from all children aged under three years old in Oxford, UK, between January 2005 and December 2018. The primary aim of this study was to assess temporal trends in the organisms

identified, and a secondary aim was to assess antimicrobial resistance patterns of Gram-negative rods in blood cultures.

Materials and Methods

Oxford University Hospitals NHS Foundation Trust (OUHNFT) is a large university teaching trust comprising of four hospitals, two of which routinely manage children, in Oxford and Banbury in southern England. There is a dedicated children's hospital at the John Radcliffe Hospital in Oxford, with approximately 100 inpatient beds, a children's emergency department, a paediatric intensive care unit (PICU), and a neonatal intensive care unit (NICU). There are approximately 9,000 births per year in the trust and the NICU also cares for complex neonatal cases transferred from across the region. At the Horton General Hospital in Banbury, there is a smaller paediatric ward, a midwife-led maternity ward, and a children's emergency department.

A list of all blood and CSF cultures taken from children under three years of age in the trust between 1st January 2005 and 31st December 2018 was retrieved from the microbiology laboratory database. Samples that were unprocessed (e.g. duplicate requests, damaged etc), or not from a clinical area within OUHNFT (e.g. research, community samples, post-mortem etc) were excluded. Results were classified by location as either neonatal units, including NICU and maternity units ('neonatal'), or paediatric areas, including PICU, the emergency department (ED), and medical and surgical wards ('paediatric'), reflecting differences in empirical prescribing guidelines in these locations. Paediatric areas were also further divided into paediatric wards and paediatric ED (including ED and adjacent clinical decision units). Infants discharged from maternity units and requiring hospital admission for possible infection within the first few days of life are usually admitted to a paediatric ward rather than the NICU. Haematology and oncology patients are also usually admitted directly to the wards rather than via ED. Results were also subclassified into the following age classes: ≤2 days; >2 to ≤28 days;

>28 days to ≤3 months; >3 to ≤6 months; >6 to ≤12 months; >12 to ≤24 months; and >24 to ≤36 months.

Samples were classified as negative if there was no growth, and positive if an organism was identified. If multiple organisms were identified in the same sample, this was considered as one sample for the temporal analysis but as separate organisms in the organisms and resistance analysis. Duplicates were excluded, and were defined as either: a) an exact duplicate (i.e. same result from the same patient on the same day) from both negative and positive samples, or b) positive samples that grew the same organism from the same patient within 7 days of a previous positive sample without an alternative intervening positive or negative result. Positive samples were further subdivided into 'pathogens' and 'common commensals'; commensals were defined using the CDC NHSN Common Commensals List 2020. [8] Samples that did not name an identified organism, e.g. 'mixed', 'environmental organisms', were also included with the 'common commensals' group.

Antimicrobial sensitivity data were extracted for Gram-negative rods on blood cultures from the microbiology database. Until 31st January 2013, susceptibility testing was done by use of disk diffusion (antibiotic disks and agar from Oxoid/Thermo Fisher Scientific Ltd, Basingstoke, UK), and since then testing has been done by microbroth dilution (BD Phoenix Automated Microbiology System, Beckton Dickinson, Franklin Lakes, NJ, USA), as described in detail elsewhere. [9]

Resistance proportions were calculated for nine commonly prescribed antimicrobials: amoxicillin, co-amoxiclav, ceftriaxone, ceftazidime, other cephalosporins (including cefalexin, cefazolin, cefepime, cefpirome, cefuroxime, cefradine), piperacillin-tazobactam, ciprofloxacin,

gentamicin and meropenem; for ‘other cephalosporins’, resistance was defined as resistance to at least one of the cephalosporins listed. Results classified as ‘intermediate’ were classified the same as ‘resistant’. As not every organism was tested against every antimicrobial (e.g. *Pseudomonas aeruginosa* was not tested for amoxicillin), resistance proportions were calculated with the number of isolates tested for each antimicrobial as the denominator. Resistance was calculated for all Gram-negative organisms, without excluding those with intrinsic resistance, as this more closely reflects what the clinician may suspect or know before full culture results may be released. Resistance pattern over time was presented after excluding organisms with intrinsic resistance to the antimicrobial, as defined by the EUCAST Intrinsic Resistance & Unusual Phenotypes guidance, to reflect changes in resistance rather than changes in the proportions of intrinsically resistant organisms. [10]

In OUHNFT, first-line empirical antimicrobials for sepsis on the neonatal units during this period were gentamicin plus benzylpenicillin (if ≤2 days old) or gentamicin plus flucloxacillin (if >2 days old); on paediatric units, first-line antimicrobials for sepsis were cefotaxime or ceftriaxone, plus amoxicillin to cover for the possibility of *Listeria monocytogenes* if <3 months old, in line with standard UK guidance. [11] [12] First-line empirical antimicrobials for suspected meningitis were cefotaxime plus amoxicillin in neonatal units and ceftriaxone alone in paediatric units. First-line empirical antimicrobial for oncology patients with febrile neutropenia was piperacillin-tazobactam.

Statistical analyses were performed on RStudio version 1.3.959 and Microsoft Excel version 16.42. This service evaluation project did not require ethical approval.

Results

Overall trends

Of 52,256 eligible blood and CSF cultures taken in the fourteen-year period, 49,298 unique samples (40,145 blood cultures and 9,153 CSF cultures) from 25,697 patients were analysed (Figure 1). 2,669 (6.7%) blood cultures and 281 (3.1%) CSF cultures grew organisms, of which 912 (34.2%, or 2.3% of total blood cultures) and 100 (35.6%, or 1.1% of total CSF cultures) samples respectively grew organisms considered pathogens (i.e. not contaminants). 25,023 (50.8%) samples were from neonatal units and 24,275 (49.2%) samples from paediatric units, of which 396 (1.6%) and 616 (2.5%) cultures respectively grew pathogens in either blood or CSF.

Figure 1. Flow diagram of samples. Numbers indicate number of samples; number of total organisms is indicated in the footnotes where appropriate.

* Represents 2,063 organisms

** Represents 228 organisms

† Represents 124 organisms

†† Represents 6 organisms

In neonatal units, the majority of samples were taken from neonates ≤2 days old, i.e. suspected early onset sepsis, for both blood (63.6%) and CSF cultures (52.3%). Numbers then rapidly declined with age (Figures 2A and C). The proportion of pathogens identified was however lowest in this age group (Figure 2B and D).

In paediatric units, there was a gradual increase with age for the number of blood cultures taken, peaking in the 12-24-month age group (Figure 2A), while the number of CSF cultures taken peaked in the 28 days-3-month age group then declined over the subsequent age groups (Figure 2C). There was no discernible difference in the proportions of pathogens for blood or CSF cultures in paediatric units (Figure 2B, 2D).

Figure 2. Age distribution of patients from which cultures were taken. A: overall numbers of blood cultures, B: proportions of pathogens in blood cultures, C: overall numbers of CSF cultures, D: proportion of pathogens in CSF cultures. D=days, m=months, BLC=blood cultures, CSFC=cerebrospinal fluid cultures. Note that the axes for A and C differ due to the much smaller numbers of CSF cultures.

Temporal trends

The total number of blood cultures taken in neonatal units showed an increase over time peaking in 2014 and then gradually declining, while in paediatric units the numbers remained static (Figure 3A). The total number of CSF cultures taken, similarly showed an increase to a peak in 2013, followed by a decline; the trend in paediatric units showed a steady increase (Figure 3C).

In contrast, the proportions positive for pathogens declined over time in blood cultures, until a trough in 2013 and 2015 in neonatal and paediatric units respectively (Figure 3B). In CSF cultures a similar decline was seen until 2015 and 2013 in neonatal and paediatric units respectively (Figure 3D).

Figure 3. Trend over time of cultures taken. A: overall numbers of blood cultures, B: proportions of pathogens in blood cultures, C: overall numbers of CSF cultures, D: proportion of pathogens in CSF cultures. BLC=blood cultures, CSFC=cerebrospinal fluid cultures. Note that the axes for A and C differ due to the much smaller numbers of CSF cultures.

Organisms

Of 980 pathogens isolated in blood cultures, 466 (47.6%) were Gram-positive bacteria; 472 (48.2%) were Gram-negative bacteria of which 426 were rods; 36 (3.7%) were yeasts; and 6 (0.6%) were *Mycobacterium* species. The most common organism was *Streptococcus agalactiae* (Group B streptococcus) (17.9%) in neonatal units and *Staphylococcus aureus* (13.4%) in paediatric units (Table 1), followed by *Enterococcus* species in both areas (17.2% and 12.2% respectively); in paediatric units, *Enterococcus* species were most commonly isolated from the general surgery ward (26.4%) and PICU (18.1%). The next common organisms were *Escherichia coli* (13.6%) and *S. aureus* (13.6%) in neonatal units and *E. coli* (12.0%) and *Streptococcus pneumoniae* (8.8%) in paediatric units. Coagulase-negative staphylococcus (CoNS) comprised the majority of common commensals (92.6% in neonatal units and 74.5% in paediatric units). Figure 4 shows the temporal distribution of the most common organisms on blood culture; Gram-positive organisms such as *S. aureus* and *Enterococcus* species both show a decline until 2014-15, followed by an increase.

Of 103 pathogens isolated in CSF cultures, 56 (54.4%) were Gram-positive bacteria; 45 (43.7%) were Gram-negative bacteria of which 39 were rods; and 2 (1.9%) were yeasts. The most common organisms in neonatal units were *S. aureus* (21.9%) and *E. coli* (18.8%), and in paediatric units were *S. pneumoniae* (15.5%) and *S. aureus* (14.1%) (Table 2); 8 of the 10 cases of *S. aureus* were found on the neuroscience/specialist surgery ward. 22 (21.4%) CSF cultures were also positive for the same organism on blood culture taken on the same day, including 81.8% of all *S. pneumoniae* (n=9/11) and *S. agalactiae* (n=9/11) isolated in CSF culture. No cases of *Listeria monocytogenes* were identified over the fourteen years. CoNS were again the most common CSF commensal (52.0% in neonatal units and 65.4% in paediatric units).

Cases of *Neisseria meningitidis*, *S. pneumoniae* and *Haemophilus influenzae* (not subtyped) in neonatal units were infrequent in blood cultures (n=0, n=3, n=2 respectively) and zero in CSF cultures. In paediatric units, *N. meningitidis* constituted 21/590 (3.6%) of positive blood cultures and 4/71 (5.6%) of positive CSF cultures; *S. pneumoniae* in 52 (8.8%) positive blood cultures and 11 (15.5%) positive CSF cultures; and *H. influenzae* in 6 (1.0%) positive blood cultures and zero CSF cultures. While numbers were small, cases of both *N. meningitidis and S. pneumoniae* in blood cultures appear to have declined over time (Figure 4).

Methicillin-resistant *Staphylococcus aureus* were isolated in 10 blood cultures and 1 CSF culture (7.4% of all *S. aureus*) from SCBU (n=6), PICU (n=1), and other paediatric wards (n=4). There were no discernible patterns over time of MRSA.

Figure 4. Temporal trends of pathogens of significance in blood cultures.

Organisms grown in blood cultures (n=3,043)					
Neonatal units (n=1,374)			**Paediatric units (n=1,669)**		
	n	**%**		**n**	**%**
Common commensals*	**984**	**71.6%**	**Common commensals****	**1079**	**64.6%**
Pathogens†	**390**	**28.4%**	**Pathogens††**	**590**	**35.4%**
Gram positive			Gram positive		
Streptococcus agalactiae (Group B)	70	17.9%	*Staphylococcus aureus*	79	13.4%
Enterococcus species *(E. faecalis, E. faecium)*	67	17.2%	*Enterococcus* species *(E. faecalis, E. faecium)*	72	12.2%
Staphylococcus aureus	53	13.6%	*Streptococcus pneumoniae*	52	8.8%
			Streptococcus agalactiae (Group B)	37	6.3%
			Streptococcus pyogenes (Group A)	16	2.7%
Gram negative			Gram negative		
Escherichia coli	53	13.6%	*Escherichia coli*	71	12.0%
Enterobacter cloacae	25	6.4%	*Pseudomonas aeruginosa*	34	5.8%
Pseudomonas aeruginosa	17	4.4%	*Enterobacter cloacae*	25	4.2%
Klebsiella oxytoca	16	4.1%	*Klebsiella pneumoniae*	22	3.7%
Serratia species *(S. marcescens, S. plymuthica)*	15	3.8%	*Neisseria meningitidis*	21	3.6%
Klebsiella pneumoniae	13	3.3%	*Moraxella* species *(M. catarrhalis, M. osloensis, M.nonliquefaciens)*	21	3.6%
Acinetobacter species *(A. baumannii, A. lwoffii, A. ursingii)*	7	1.8%	*Acinetobacter* species *(A. baumannii, A. lwoffii, A. ursingii, A. junii, A. parvus)*	20	3.4%
Citrobacter species *(C. freundii, C. koseri)*	5	1.3%	*Klebsiella oxytoca*	19	3.2%
			Haemophilus influenzae	6	1.0%
			Pseudomonas species (*P. oryzihabitans, P. lutoela, P. fluorescens, P. putida, P. stutzeri)*	6	1.0%
			Klebsiella aerogenes	5	0.8%
			Citrobacter species *(C. freundii, C. koseri)*	5	0.8%
			Stenotrophomonas maltophilia	5	0.8%
			Serratia species (S. marcescens, S. liquefaciens)	4	0.7%
Other			Other		
Candida albicans	13	3.3%	*Candida albicans*	8	1.4%
Candida glabrata	5	1.3%	*Mycobacterium* species	6	1.0%
Candida species *(C. lusitaniae, C. parapsilosis)*	4	1.0%	*Candida parapsilosis*	4	0.7%

Table 1. Organisms in blood cultures, stratified by location. Organisms grouped by genus have the individual species, if named, in brackets. % are expressed as a proportion of the total number of pathogens per location. Organisms with fewer than 3 isolates are listed in the footnotes below with number indicated in brackets.

* most common: coagulase-negative staphylococci (CoNS) (n=911), viridans streptococci (n=26), *Micrococcus* species (n=18), diphtheroids (n=13).

** most common: CoNS (n=804), viridans streptococci (n=134), *Micrococcus* species (n=38), diphtheroids (n=36).

† Other organisms included Gram-positives: *Streptococcus pneumoniae* (n=3), *Lactococcus lactis* (n=2), *Lactobacillus paracasei*, *Lysinibacillus* species, *Streptococcus pyogenes* (Group A) *(n=1 each)*

Gram-negatives: *Moraxella* species *(M. osloensis), Klebsiella aerogenes* (n=3 each), *Brevundimonas* species, *Haemophilus influenzae*, *Morganella morganii*, *Stenotrophomonas maltophilia* (n=2 each), *Haemophilus sputorum, Proteus mirabilis, Providencia stuartii, Pseudomonas* species (n=1 each)

Other: Yeasts (n=1)

†† Other organisms included Gram-positives: Nutritionally variant streptococci (n=3), *Lactococcus lactis, Streptococcus dysgalactiae (Group C/G)* (n=2 each), *Bifidobacterium* species, *Clostridium tertium, Gemella morbillorum, Lactobacillus* species, *Lysinibacillus fusiformis* (n=1 each)

Gram-negatives: *Achromobacter* species *(A. dentrificans, A. xylosoxidans), Kluyvera species, Proteus mirabilis* (n=3 each), *Enterobacter* species *(E. ludwigii), Aeromonas* species, coliforms, *Delftia acidovorans*, *Escherichia* species *(E. fergusonii, E. hermanii), Pantoea* species, *Rhizobium radiobacter, Salmonella* species, *Sphingomonas paucimobilis* (n=2 each), *Eikenella corrodens, Haemophilus parainfluenzae, Haemophilus* species, *Hafnia alvei, Massilia timonae, Ochrobactrum anthropi, Paracoccus yeei, Proteus* species, *Rhizobium radiobacter, Salmonella paratyphi A, Stenotrophomonas acidaminiphila, Cronobacter sakazakii* (n=1 each)

Other: *Paecilomyces* species (n=1)

Organisms grown in CSF cultures (n=331)					
Neonatal units (n=130)			**Paediatric units (n=201)**		
	n	**%**		**n**	**%**
Common commensals*	**98**	**75.4%**	**Common commensals****	**130**	**64.7 %**
Pathogens†	**32**	**24.6%**	**Pathogens††**	**71**	**35.3 %**
Gram positive			Gram positive		
Staphylococcus aureus	7	21.9%	*Streptococcus pneumoniae*	11	15.5%
Enterococcus species *(E. faecium, other)*	4	12.5%	*Staphylococcus aureus*	10	14.1%
Streptococcus agalactiae (Group B)	3	9.4%	*Streptococcus agalactiae (Group B)*	8	11.3%
			Enterococcus species (*E. faecalis, other)*	8	11.3%
Gram negative			Gram negative		
Escherichia coli	6	18.8%	*Escherichia coli*	8	11.3%
			Acinetobacter lwoffii	7	9.9%
			Neisseria meningitidis	4	5.6%

Table 2. Organisms in CSF cultures, stratified by location. Organisms grouped by genus have the individual species, if named, in brackets. % are expressed as a proportion of the total number of pathogens per location. Organisms with fewer than 3 isolates are listed in the footnotes below with number indicated in brackets.

* most common: CoNS (n=51), viridans streptococci (n=33).

** most common: CoNS (n=85), viridans streptococci (n=27).

† Other organisms included Gram-positives*: Gemella morbillorum, Leuconostoc* species (n=1 each)

Gram-negatives: *Pseudomonas* species *(P. stutzeri, P. oryzihabitans), Acinetobacter lwoffii, Pseudomonas aeruginosa* (n=2 each), *Brevundimonas vesicularis, Klebsiella oxytoca* (n=1 each)

Other: *Candida albicans* (n=2)

†† Other organisms included Gram-positives: *Gemella morbillorum, Lactobacillus rhamnosus, Streptococcus pyogenes (Group A*) (n=1 each)

Gram-negatives: *Enterobacter cloacae, Pseudomonas* species *(P. luteola, P. stutzeri)* (n=2 each), *Enterobacter* species, *Escherichia fergusonii, Klebsiella oxytoca, Haemophilus parainfluenzae, Moraxella catarrhalis, Ochrobactrum anthropi, Pseudomonas aeruginosa, Veillonella* species (n=1 each)

Antimicrobial resistance of Gram-negative rods in blood cultures

Of 426 blood cultures with Gram-negative rods isolated, antimicrobial resistance data was available for 413 (97.0%) (Table 3). Figure 5 shows the proportion of isolates that are resistant to two antimicrobials, i.e. where there may be an addition or a switch of an antimicrobial if the patient was not improving or deteriorating.

Overall, in neonatal units, resistance to gentamicin was found in 9.3% (n=15/161), which consisted of *Escherichia coli* (n=8), *Klebsiella* (n=5) species and *Stenotrophomonas maltophilia* (n=2). Resistance was lower to other antibiotics that may be used in late-onset neonatal sepsis such as ciprofloxacin (6.2%) and meropenem (1.3%). Isolates that were resistant to both gentamicin and other antibiotics were lower, including ceftriaxone (4.7%), piperacillin-tazobactam (5.0%), ciprofloxacin (4.3%), and meropenem (0.6%).

In paediatric units, resistance in the wards were overall higher than in ED for all the penicillin and cephalosporin antibiotics, but comparable for ciprofloxacin and gentamicin. Resistance to ceftriaxone was found in 17.1% (6/35) in ED and 25.8% (51/198) on the wards; in combination with gentamicin the proportion resistant was 3.1% (both paediatric areas combined). *Pseudomonas aeruginosa* (n=21) (intrinsic resistance), *E. coli* (n=6), and *Enterobacter cloacae* (n=5) were the most common species resistant to ceftriaxone. Resistance to amoxicillin was high, at 41.9% in ED and 81.9% in the wards. Resistance to piperacillin-tazobactam was 2.8% in ED and 13.9% in the wards, but isolates that were additionally resistant to gentamicin or ciprofloxacin were 2.5% and 1.3% respectively (both paediatric areas combined).

2.0% (8/400) of all Gram-negative rods across both units were resistant to meropenem, which were *Pseudomonas aeruginosa* (n=5) and *Stenotrophomonas maltophilia* (n=1) on paediatric

wards, and *Acinetobacter baumannii* (n=1) and *Stenotrophomonas maltophilia* (n=1) in neonatal units.

	Neonatal (n=163)		Paediatric ED (n=39)		Paediatric wards (n=211)		Overall (n=413)	
	Tested	**% R**	**Tested**	**% R**	**Tested**	**% R**	**Tested**	**% R**
Amoxicillin	143	81.1%	31	41.9%	177	81.9%	351	78.1%
Co-amoxiclav	151	45.7%	36	19.4%	192	47.9%	379	44.3%
Ceftriaxone	150	21.3%	35	17.1%	198	25.8%	383	23.2%
Ceftazidime	157	14.0%	32	6.3%	194	10.8%	383	11.7%
Other cephalosporins	98	55.1%	16	18.8%	155	58.1%	269	54.6%
Piperacillin-tazobactam	160	11.9%	36	2.8%	201	13.9%	397	12.1%
Ciprofloxacin	162	6.2%	39	7.7%	204	5.9%	405	6.2%
Gentamicin	161	9.3%	37	8.1%	203	6.9%	401	8.0%
Meropenem	160	1.3%	38	0.0%	202	3.0%	400	2.0%

Table 3. Resistance in Gram-negative rods in blood cultures to commonly used antimicrobials, stratified by location. %R = percentage resistant, calculated as number of resistant isolates as the numerator and the number tested to that antimicrobial ('tested') as the denominator. ED = emergency department.

Figure 5. Co-resistance of Gram-negative rods on blood cultures, shaded by level of resistance. Amox = amoxicillin, Coamox = co-amoxiclav, Ceftri = ceftriaxone, Ceftaz = ceftazidime, Ceph = other cephalosporins, Piptaz = piperacillin-tazobactam, Cipro = ciprofloxacin, Gent = gentamicin, Mero = meropenem. A: neonatal units, B: paediatric units (including paediatric wards and paediatric ED). For example, in neonatal units 5.3% were resistant to both co-amoxiclav and gentamicin.

Figure 6 shows time trends in resistance for selected antimicrobials, after removing organisms with intrinsic resistance. Resistance to amoxicillin decreased over time, while there were few discernible trends for other antimicrobials.

Resistance observed in selected Gram-negative rods are summarised in Table 4, excluding those antibiotics to which the organisms have intrinsic resistance. 18/199 (9.0%) of *E. coli* and *Klebsiella* isolates were ESBL-producing. In *E. coli*, resistance was higher in paediatric wards compared to paediatric ED for all antibiotics except ciprofloxacin, and in *Klebsiella*, all isolates from ED were susceptible to all antibiotics. Of 51 *Pseudomonas aeruginosa* isolates, only 1 was resistant to ceftazidime, which was also resistant to piperacillin-tazobactam and meropenem; this isolate was from PICU and the first blood culture taken in that admission. No *Pseudomonas* species were resistant to ciprofloxacin or gentamicin.

Figure 6. Temporal trends of resistance to selected antimicrobials of Gram-negative rods in blood cultures. For other cephalosporins, 2015-2018 is removed as they were seldomly tested after 2015. Organisms that were intrinsically resistant to the antimicrobial were removed.

	E. coli				*Klebsiella*				*Enterobacter*			
	Neonatal (n=53)	**Paediatric ED (n=21)**	**Paediatric wards (n=51)**	**Total (n=125)**	**Neonatal (n=32)**	**Paediatric ED (n=3)**	**Paediatric wards (n=39)**	**Total (n=74)**	**Neonatal (n=23)**	**Paediatric ED (n=1)**	**Paediatric wards (n=25)**	**Total (n=49)**
Amoxicillin	56.6%	38.1%	64.7%	56.8%	NA	NA	NA	NA	NA	NA	NA	NA
Co-amoxiclav	26.4%	14.3%	33.3%	27.2%	15.6%	0.0%	18.0%	16.2%	NA	NA	NA	NA
Ceftriaxone	19.6%	4.8%	10.0%	13.1%	21.9%	0.0%	18.0%	18.9%	13.0%	100.0%	16.0%	16.3%
Ceftazidime	13.2%	4.8%	9.8%	10.4%	21.9%	0.0%	12.8%	16.2%	8.7%	100.0%	12.0%	12.2%
Other cephalosporins	41.9%	9.1%	43.2%	38.4%	24.0%	0.0%	31.3%	27.1%	NA	NA	NA	NA
Piperacillin-tazobactam	9.4%	4.8%	11.8%	9.6%	15.6%	0.0%	28.2%	21.6%	8.7%	0.0%	8.0%	8.2%
Ciprofloxacin	9.4%	4.8%	2.0%	5.6%	9.4%	0.0%	2.6%	5.4%	0.0%	0.0%	4.0%	2.0%
Gentamicin	15.1%	0.0%	7.8%	9.6%	15.6%	0.0%	5.1%	9.5%	0.0%	100.0%	8.0%	6.1%
Meropenem	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 4. Resistance in selected Gram-negative rods on blood cultures to commonly used antimicrobials, stratified by location. %R = percentage resistant, calculated as number of resistant isolates as the numerator and the number tested (not shown for clarity) to that antimicrobial as the denominator. NA = not applicable, as the organism is intrinsically resistant to the antimicrobial.

Discussion

We describe here the trends of paediatric blood and CSF cultures over fourteen years from a major NHS trust in England. The number of cultures taken per year generally increased over time for the first 9-10 years of the study period, with a corresponding decline in culture-positive proportions; this pattern is particularly observed in the common Gram-positive organisms *S. aureus* and *Enterococcus* species. The reason for this may be that there are an increasing number of comparatively well children being seen over time; it may also reflect a lower threshold to culture, i.e. taking cultures with a low clinical suspicion of bloodstream or CSF infection. This disparity is most pronounced in children less than 2 days old in neonatal units, where the culture-positive proportion was lowest despite two-thirds of all blood cultures being taken from this group. The threshold to culture may be particularly low in this population, as also reflected in the updated neonatal infection NICE guidelines that suggest a more aggressive approach to investigating and treating early onset neonatal sepsis. [11]

The most common organisms isolated across both units were *S. aureus*, *Enterococcus* species and *E. coli*, plus Group B streptococci in neonatal units, and *S. pneumoniae* in paediatric units. The distribution of organisms is broadly similar to other UK studies in both neonates [13 14 15] and paediatric patients [16 17 18 19], although direct comparisons are often difficult given the variability in defining contaminants, locations and age categories. Cases of *N. meningitidis*, *S. pneumoniae* and *H. influenzae* (no typing done) were very few in our study, with a decline over time for *N. meningitidis* and *S. pneumoniae.* This follows the trends found in previous UK studies showing a substantial decline in these vaccine-preventable organisms over time. [18 20 21] We found just 11 cases of MRSA over 14 years, mostly in SCBU or PICU, consistent with findings that overall MRSA is rare in children and focused in the very young or those with a history of invasive interventions. [22]

Overall, three-quarters of all Gram-negative rods were resistant to amoxicillin, more than half to first and second generation cephalosporins, and 44% to co-amoxiclav. Resistance to other frequently used first-line agents such as third-generation cephalosporins or piperacillin-tazobactam ranged from 11-23%, whereas resistance to ciprofloxacin and gentamicin were low (6-8%). Resistance to meropenem was rare, and no organisms were carbapenem-resistant Enterobacteriaceae (CRE). 9% of *E. coli* and *Klebsiella* species were ESBL-producing organisms.

Neonatal units are often areas of high selection pressure from intense antimicrobial prescribing, with one study finding that 61% of patients in NICU received an antimicrobial. [23] The OUHNFT guidelines are in line with the updated NICE guidance on early-onset sepsis (<72 hours), which suggest benzylpenicillin and gentamicin as empiric treatment, but also recommends the addition of cefotaxime if meningitis is suspected. [11] In our study, resistance in Gram-negative rods to gentamicin and ceftriaxone in neonatal units were 9.3% and 21.3% respectively, but only 4.7% were resistant to both. Antimicrobial resistance rates in neonatal units are broadly similar to other studies across the country, which report around 8-31% resistance to empirical therapies in neonates over 2 days of age. [13 14]

On paediatric units, resistance was generally higher on the wards compared to ED, where the patient cohort is more likely to be antibiotic-naive than the inpatient population, especially as haematology and oncology patients, who are usually exposed to multiple antibiotic courses, are often admitted directly to the ward. The OUHNFT guidelines in paediatric areas are the same as the current NICE guidance for fever under 5s and the NICE sepsis guidelines, which suggests ceftriaxone plus ampicillin or amoxicillin if the patient is <3 months old, and

piperacillin-tazobactam for febrile neutropenic oncology patients. [12] [24] [25] In our study, ceftriaxone resistance in Gram-negative rods was 25.8% in paediatric wards and 17.1% in paediatric ED (including organisms intrinsically resistant to ceftriaxone such as *Pseudomonas* spp.); however, only 2-4% of organisms were resistant to both ceftriaxone and alternative agents such as ciprofloxacin, gentamicin or meropenem, suggesting that the addition and/or switch to these agents may help improve coverage in patients who are not improving or are deteriorating. Further, no culture-positive cases of *Listeria monocytogenes* were identified over fourteen years. A previous study found that 97% of all *L. monocytogenes* cases reported to Public Health England were in younger children less than 30 days old, and thus proposed a revision of the NICE guidelines to only include amoxicillin in those <30 days old rather than <3 months. [26] Our study provides a further viewpoint that *Listeria* infection is rare in young infants.

Empirical therapy is often prescribed based on clinical suspicion of Gram-negative sepsis, or on initial blood culture results of Gram-negative rods before the full result is available. Our data, which summarises resistance in all Gram-negative rods by clinical area, therefore provide valuable evidence to help inform the clinician on the choice of initial antimicrobials. As none of the empiric antimicrobial regimens in either area provide complete coverage, it is essential for clinicians to regularly assess children and consider alteration of the antimicrobial regimen if there is deterioration or no improvement. Our data also show which alternative antimicrobials may be more likely to be effective.

Given significant regional variation, understanding the epidemiology and AMR trends of common bacterial infections at a local level is key to developing evidence-based empirical therapies. A significant strength of our study is that we present longitudinal microbiology data

for all infants and children from a large hospital trust, which can be fed back directly to inform local clinical practice. There are, however, some limitations. We did not collect clinical data, so it was not possible to comment on the clinical relevance of the culture results or gain further demographic detail such as prematurity or co-morbidities; we also did not distinguish community- or hospital-acquired infections, nor patients who had multiple admissions who may have different patterns of infection and/or resistance. Moreover, we also did not perform sub-analyses by specific departments, but there is evidence that haematology-oncology wards and PICU may have different patterns of antimicrobial resistance compared with other paediatric areas. [7] Finally, microbiological practices including antimicrobial sensitivity testing varied over the years, such that denominators differed when calculating resistance. More robust surveillance data that is linked to clinical and treatment data can further inform rational antimicrobial prescribing going forward.

Conclusion

In conclusion, we have shown an increasing number of infants investigated in the form of blood and CSF cultures, but a decline in positive cultures over time. Resistance to empirical antimicrobials were seen in all sites suggesting that consideration should be given to escalating therapy if the child is not improving on empiric treatment or there is a culture positive for a Gram-negative organism prior to sensitivity results. This study provides valuable evidence to inform locally tailored antimicrobial prescribing guidelines and adds to the paediatric literature on antimicrobial resistance. Studies such as this can be used in future to feed back on the effectiveness of local antimicrobial stewardship initiatives.

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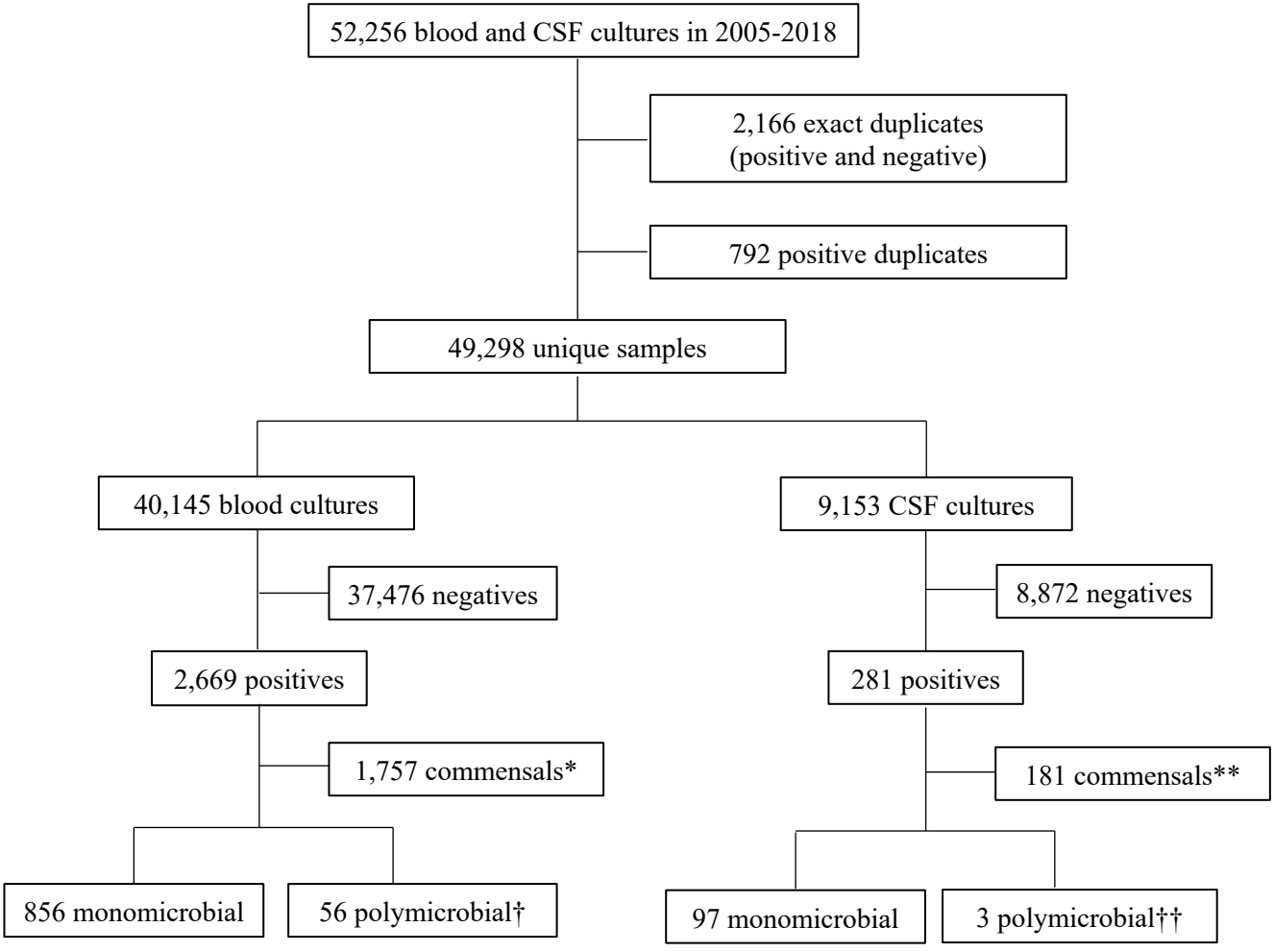
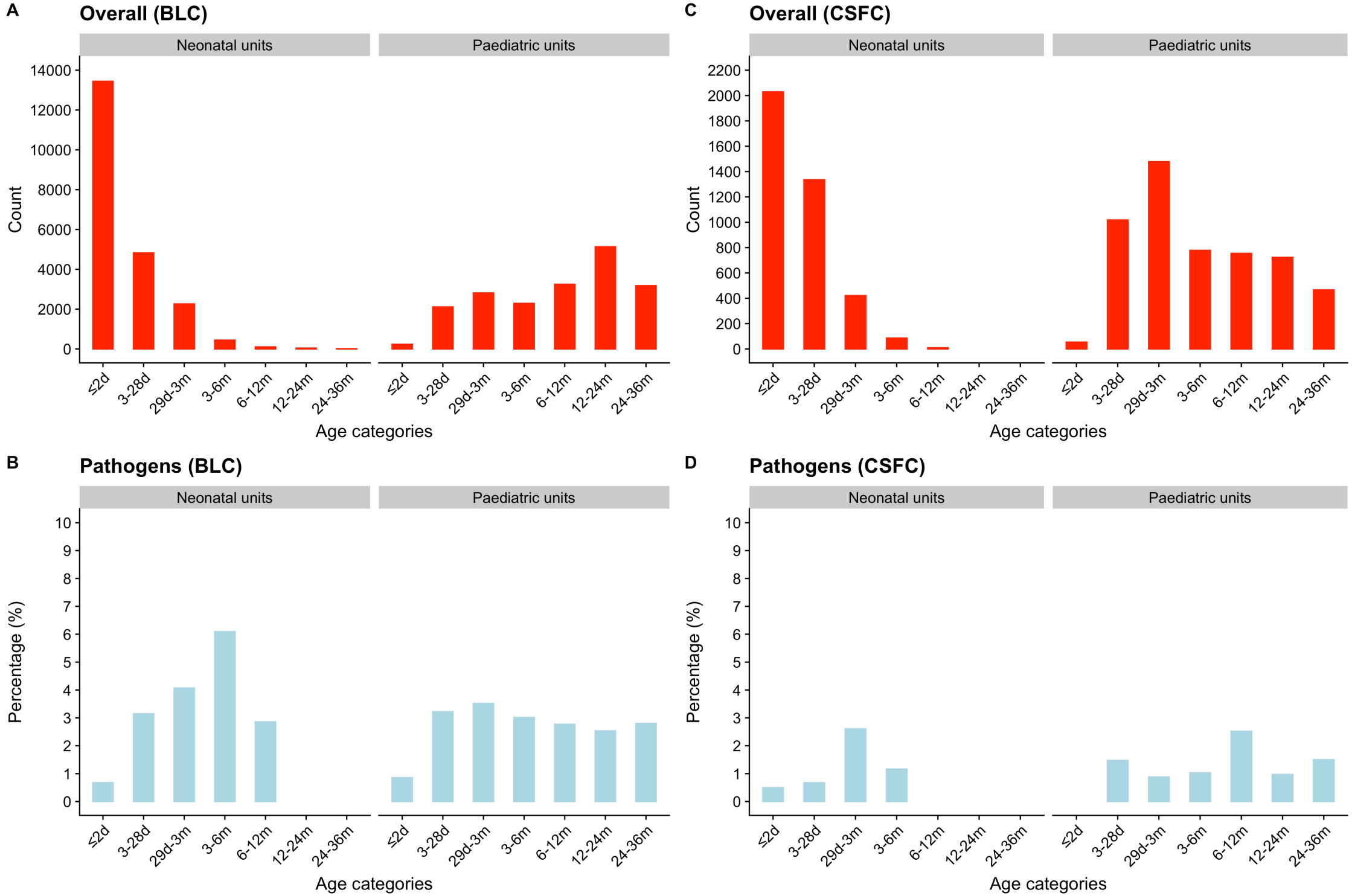
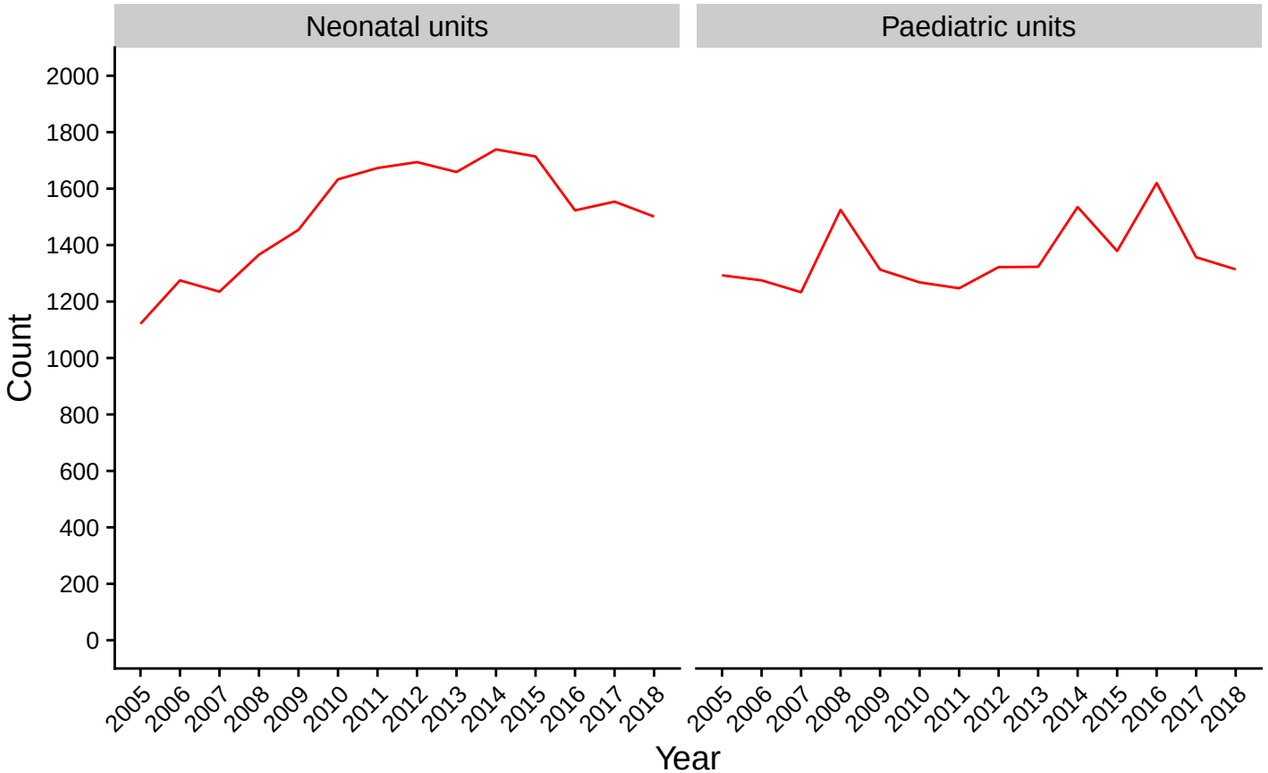

52,256 blood and CSF cultures in 2005-2018
2,166 exact duplicates (positive and negative)
792 positive duplicates
49,298 unique samples
40,145 blood cultures
9,153 CSF cultures
37,476 negatives
8,872 negatives
2,669 positives
281 positives
1,757 commensals*
181 commensals**
856 monomicrobial
56 polymicrobial†
97 monomicrobial
3 polymicrobial††


A
Overall (BLC)
Neonatal units
Paediatric units
Count
14000
12000
10000
8000
6000
4000
2000
0
≤2d
3-28d
29d-3m
3-6m
6-12m
12-24m
24-36m
Age categories
C
Overall (CSFC)
Neonatal units
Paediatric units
Count
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
≤2d
3-28d
29d-3m
3-6m
6-12m
12-24m
24-36m
Age categories
B
Pathogens (BLC)
Neonatal units
Paediatric units
Percentage (%)
10
9
8
7
6
5
4
3
2
1
0
≤2d
3-28d
29d-3m
3-6m
6-12m
12-24m
24-36m
Age categories
D
Pathogens (CSFC)
Neonatal units
Paediatric units
Percentage (%)
10
9
8
7
6
5
4
3
2
1
0
≤2d
3-28d
29d-3m
3-6m
6-12m
12-24m
24-36m
Age categories

A

Overall (BLC)



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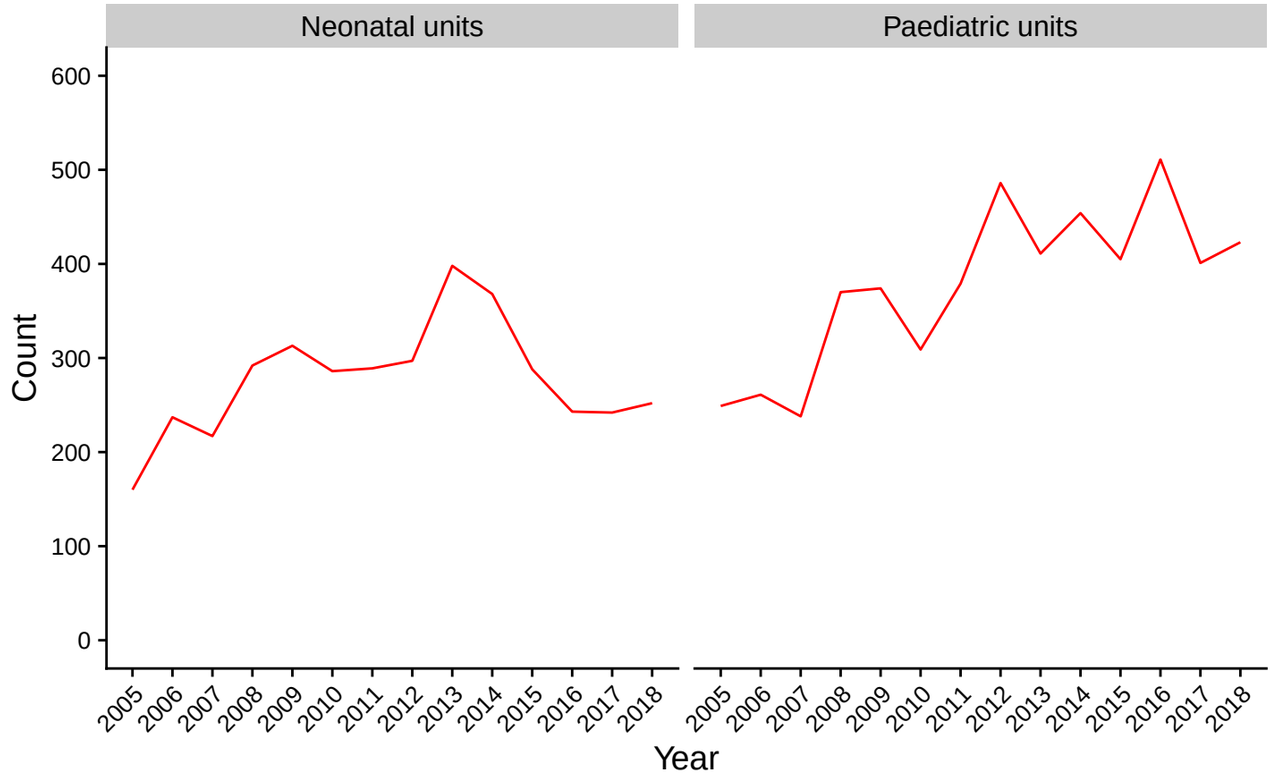

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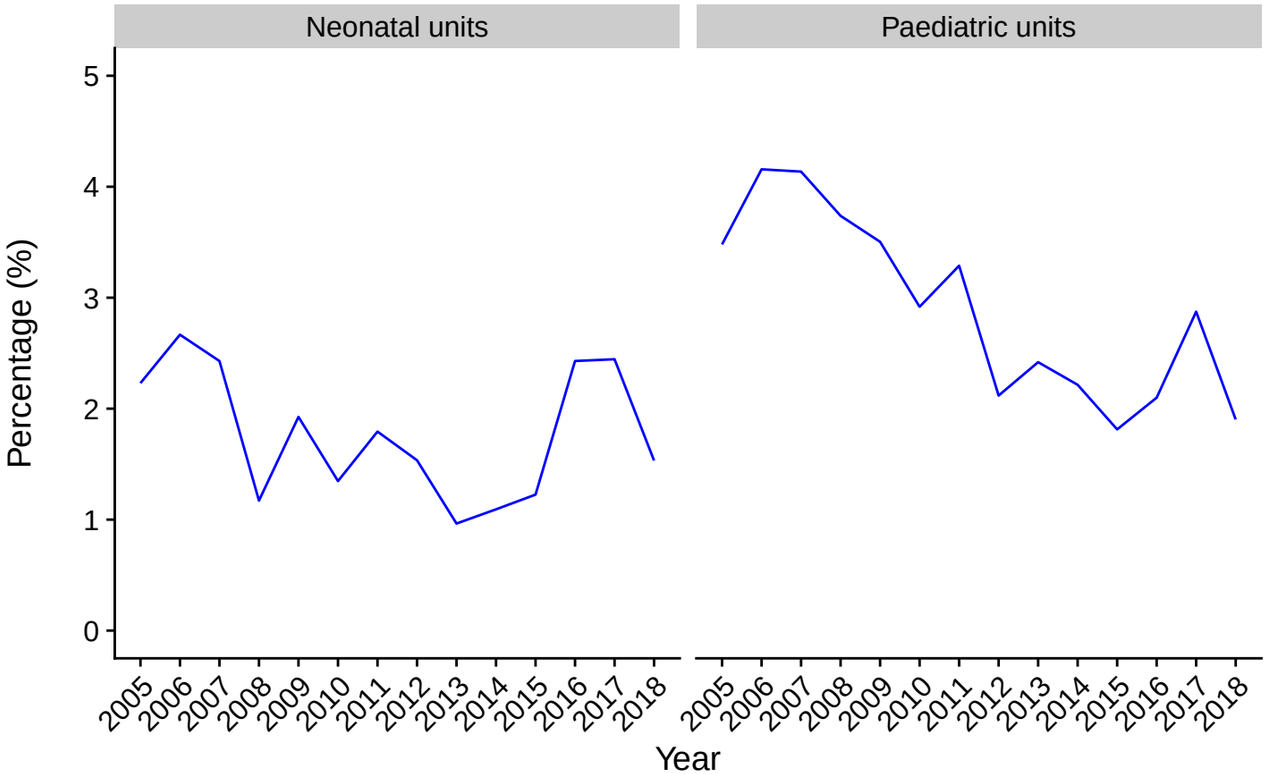

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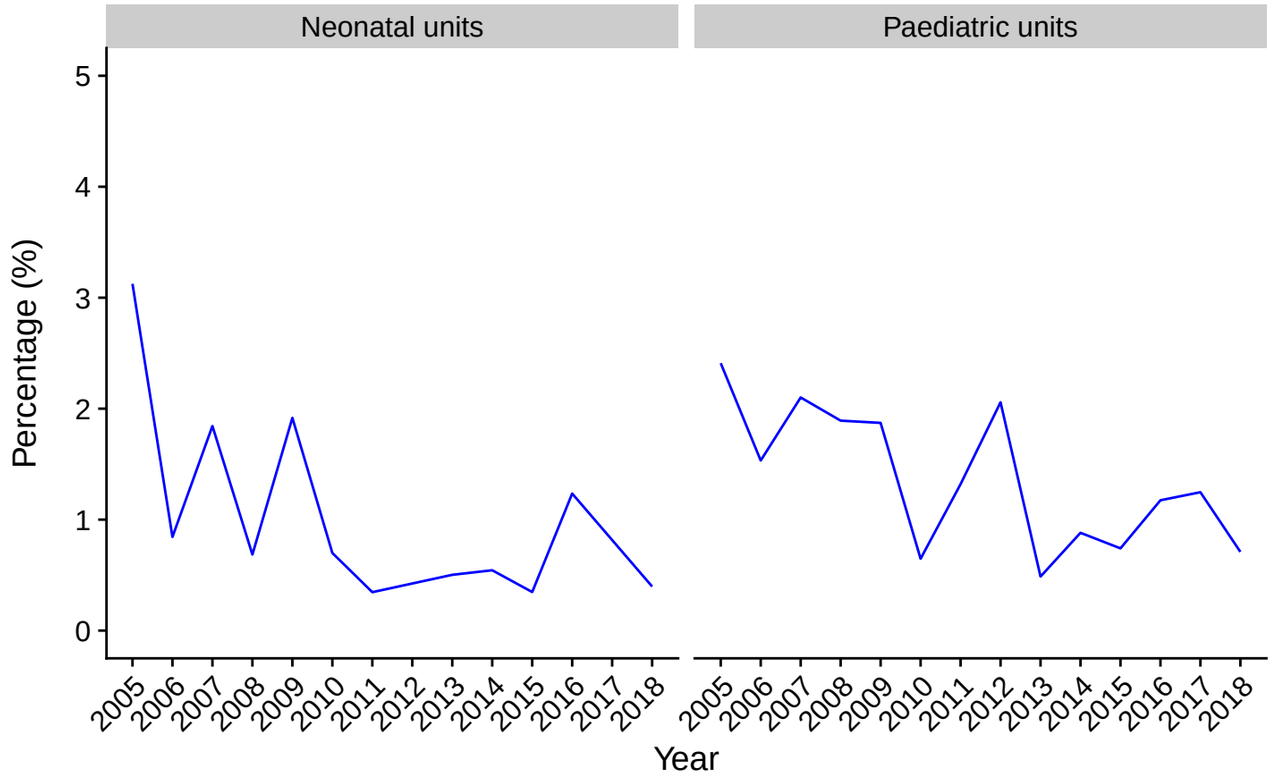
Pathogens (CSFC)



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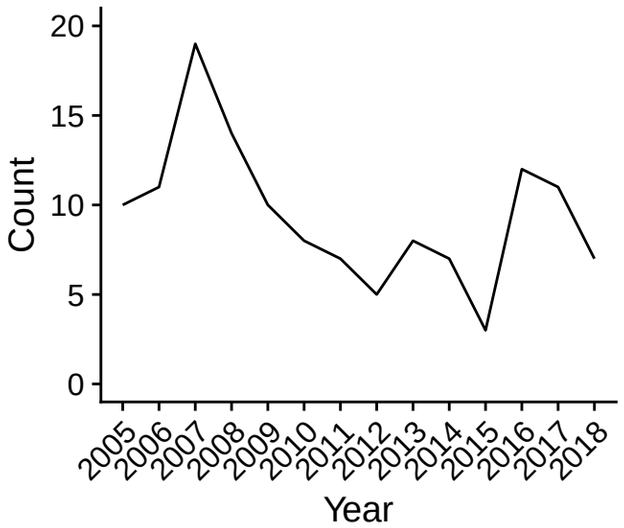
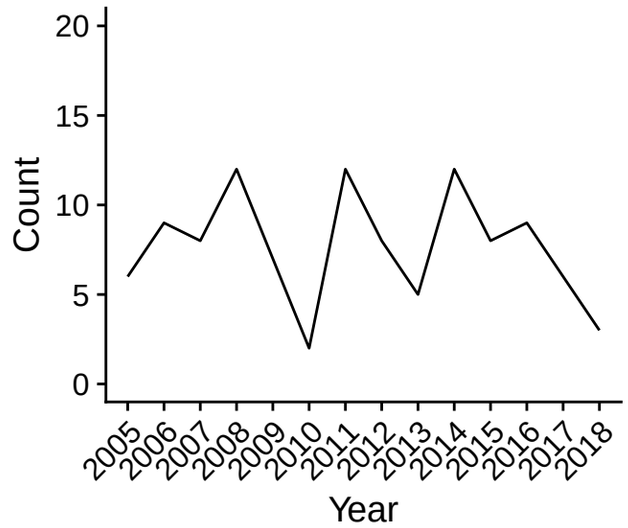
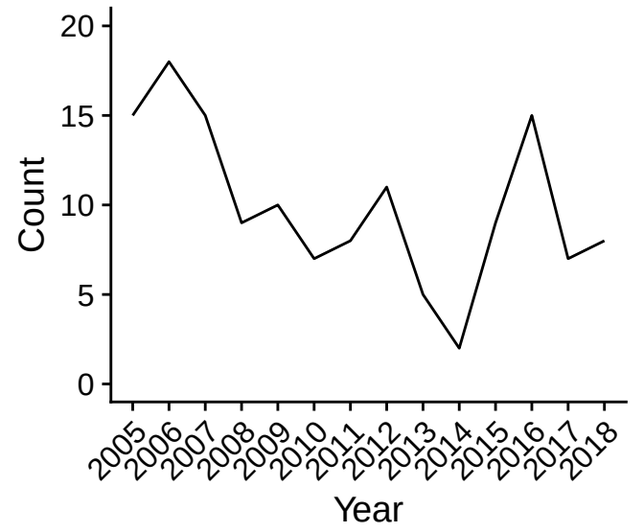

Staphylococcus aureus
Count
Year

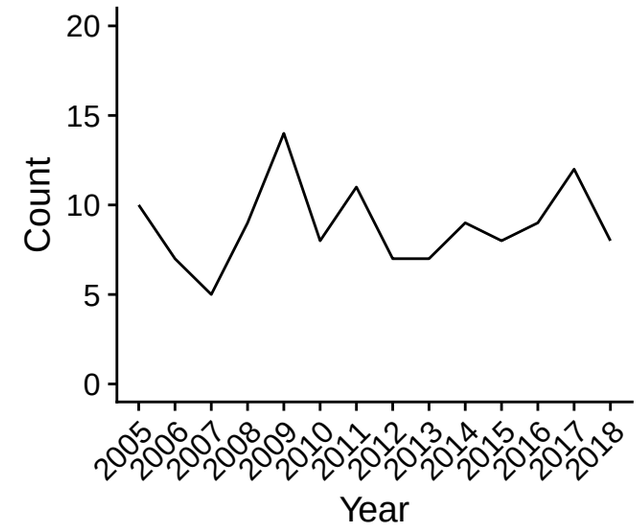
B


Streptococcus agalactiae
Count
Year

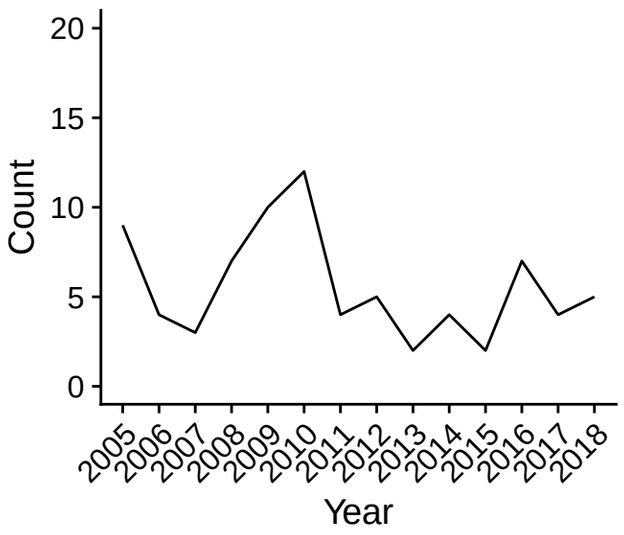
C


Enterococcus species
Count
Year

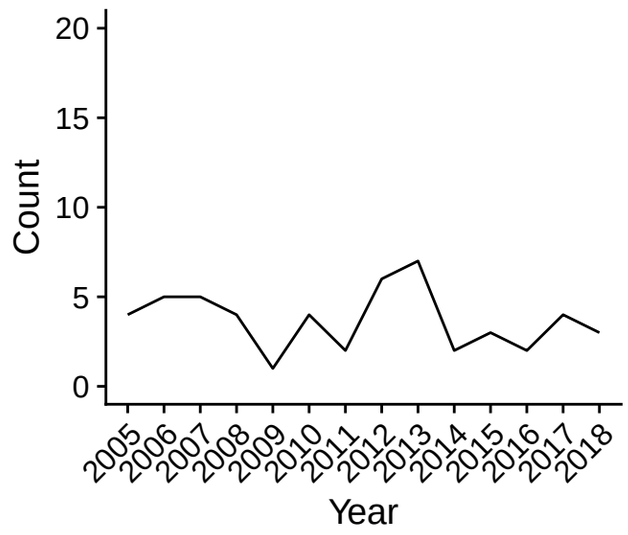
D


Escherichia coli
Count
Year

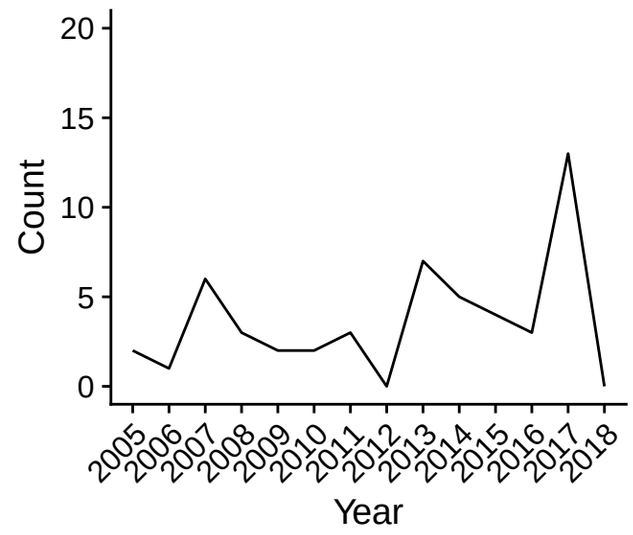
E


Klebsiella species
Count
Year

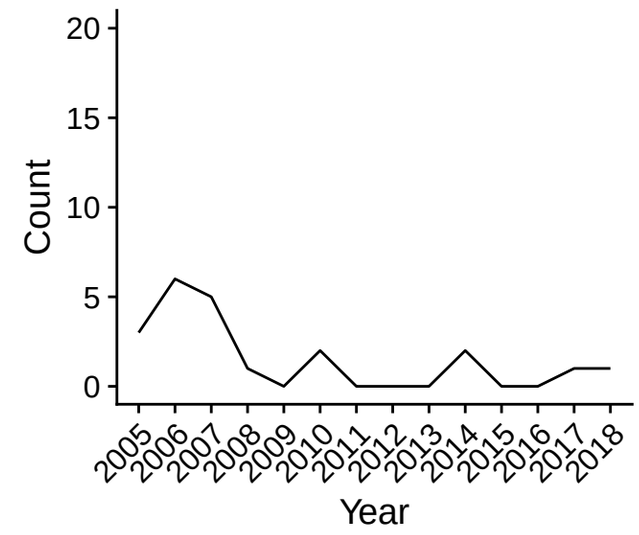
F


Enterobacter species
Count
Year

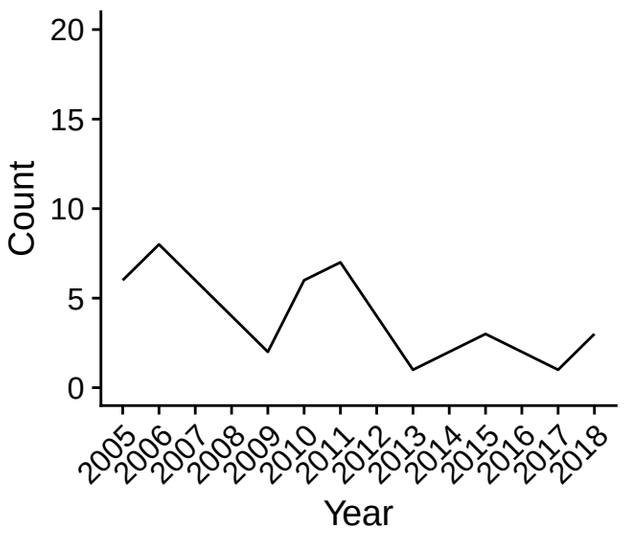
G


Pseudomonas aeruginosa
Count
Year

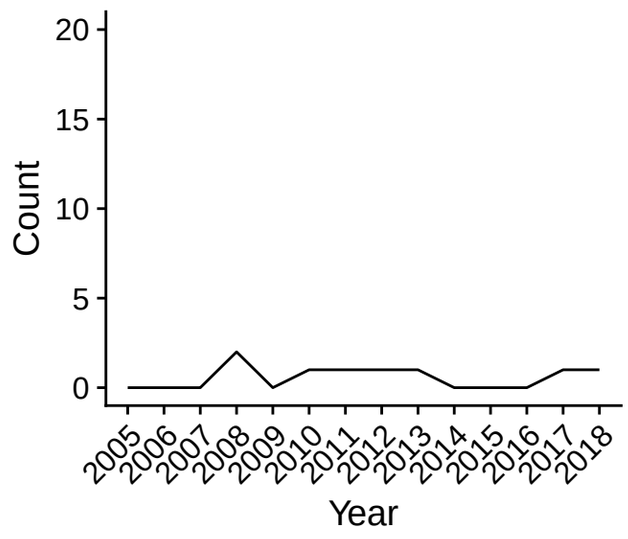
H

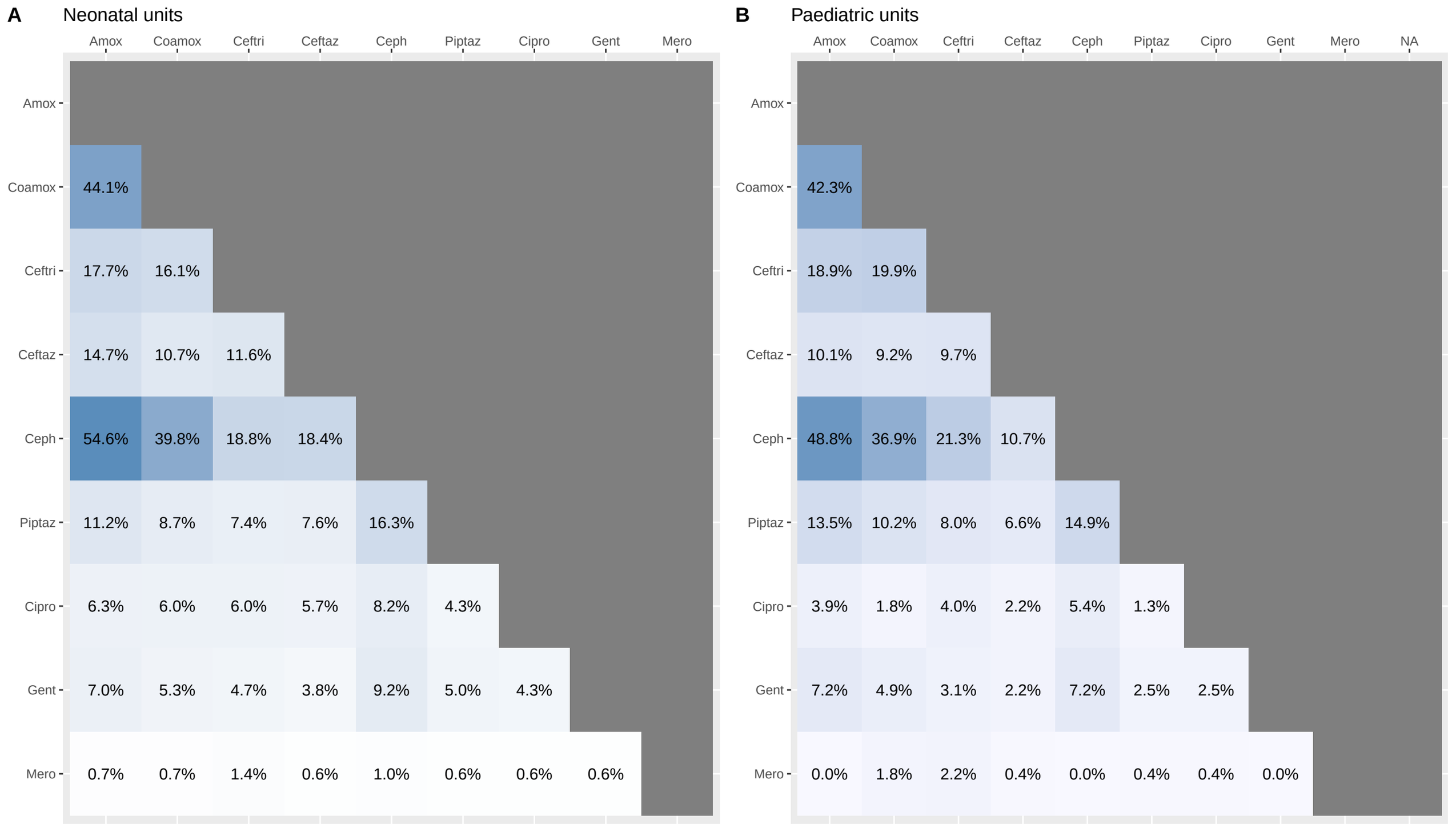

Neisseria meningitidis
Count
Year

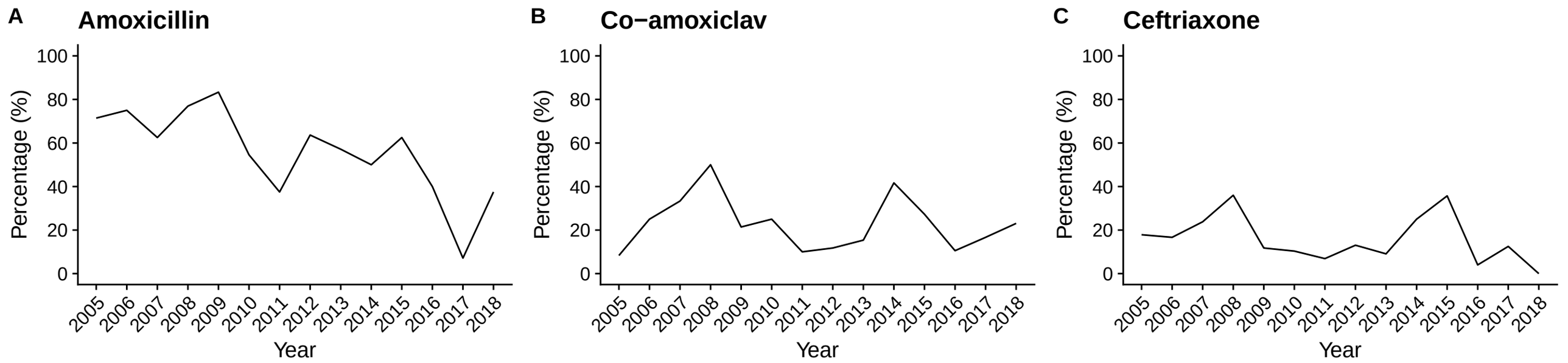
I


Streptococcus pneumoniae
Count
Year

J


Haemophilus influenzae
Count
Year


A
Neonatal units
Amox
Coamox
Ceftri
Ceftaz
Ceph
Piptaz
Cipro
Gent
Mero
44.1%
17.7% 16.1%
14.7% 10.7% 11.6%
54.6% 39.8% 18.8% 18.4%
11.2% 8.7% 7.4% 7.6% 16.3%
6.3% 6.0% 6.0% 5.7% 8.2% 4.3%
7.0% 5.3% 4.7% 3.8% 9.2% 5.0% 4.3%
0.7% 0.7% 1.4% 0.6% 1.0% 0.6% 0.6% 0.6%
B
Paediatric units
Amox
Coamox
Ceftri
Ceftaz
Ceph
Piptaz
Cipro
Gent
Mero
NA
42.3%
18.9% 19.9%
10.1% 9.2% 9.7%
48.8% 36.9% 21.3% 10.7%
13.5% 10.2% 8.0% 6.6% 14.9%
3.9% 1.8% 4.0% 2.2% 5.4% 1.3%
7.2% 4.9% 3.1% 2.2% 7.2% 2.5% 2.5%
0.0% 1.8% 2.2% 0.4% 0.0% 0.4% 0.4% 0.0%


A
Amoxicillin
B
Co−amoxiclav
C
Ceftriaxone
D
Ceftazidime
E
Other cephalosporins
F
Piperacillin−tazobactam
G
Gentamicin
H
Ciprofloxacin
I
Meropenem
Percentage (%)
Year
0
20
40
60
80
100
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018