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Laboratory surveillance of *Pseudomonas* and *Stenotrophomonas* spp bacteraemia in England, Wales and Northern Ireland: 2017

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Laboratory surveillance of *Pseudomonas* spp. and Stenotrophomonas spp. bacteraemia in England, Wales and Northern Ireland: 2017

These analyses are based on data relating to bloodstream infections caused by *Pseudomonas* spp. and *Stenotrophomonas* spp. reported by laboratories between 2009 and 2017. Data for England were extracted on 17 May 2018 from Public Health England's (PHE) voluntary surveillance database, the Second Generation Surveillance System (SGSS). Data for Wales and Northern Ireland were extracted separately (DataStore on 12 Feb 2018 and CoSurv on 15 June 2018, respectively) for inclusion in the geographical and species analyses.

Rates of laboratory reported bacteraemia were calculated using mid-year resident population estimates for the respective year and geography with the exception of 2017 rates, which were based on 2016 population estimates as population estimates for 2017 were not available at the time of producing this report [1]. Geographical analyses were based on the residential postcode of the patient if known (otherwise the GP postcode if known or failing that the postcode of the laboratory) with cases in England being assigned to one of nine local PHE centres (PHECs) formed from administrative local authority boundaries.

The report includes analyses on the trends, age and sex distribution and geographical distribution of cases of *Pseudomonas* spp. and *Stenotrophomonas* spp. bacteraemia in England, Wales and Northern Ireland. Single-agent antimicrobial susceptibility trends since 2015 are reported for England and Northern Ireland based on SGSS AMR and CoSurv data, respectively. Multi-drug antimicrobial resistance trends since 2015 are reported for England, based on SGSS AMR data. A <u>web appendix is available</u> featuring the findings of this report, including data submitted via SGSS from laboratories in England. The data presented here may differ from data in previous publications due to inclusion of late reports.

Key points

- the rate of *Pseudomonas* spp. bacteraemia in England, Wales and Northern Ireland increased by 15.7% (from 7.0 to 8.1 reports per 100,000 population) between 2009 and 2017, and by 26.6% (from 6.4 to 8.1 reports per 100,000 population) between 2013 and 2017.
- the combined Stenotrophomonas spp. bacteraemia rate in England, Wales and Northern Ireland remained constant at 0.8 reports per 100,000 population between 2013 and 2017, although the rate in Wales increased by 85.6% from 0.7 to 1.3 reports per 100,000 during this period.
- in England between 2013 and 2017, the highest increases in *Pseudomonas* bacteraemia rate were observed in the North East (51.9%; 5.4 to 8.2 reports per 100,000 population) and the South East (35.8%; 6.7 to 9.1 reports per 100,000 population) PHE Centres.
- in 2017, the highest rate of Stenotrophomonas bacteraemia in England was observed in the North East and North West PHE Centres, at 1.0 and 0.9 reports per 100,000 population, respectively.
- in 2017, *Pseudomonas aeruginosa* continued to be the most commonly isolated *Pseudomonas* species accounting for approximately 82% of all blood isolates of this genus.
- in 2017, the highest *Pseudomonas* bacteraemia rates were observed in patients over the age of 75 years (63.6 and 24.2 reports per 100,000 population in men and women, respectively)
- the highest rate of *Stenotrophomonas* bacteraemia was observed amongst men and women between the ages of 65-74, with 2.1 and 1.4 reports per 100,000 population respectively.
- between 2015 and 2017, resistance patterns for *Pseudomonas aeruginosa* and
 Stenotrophomonas for key antimicrobial agents remained broadly stable.

Trends

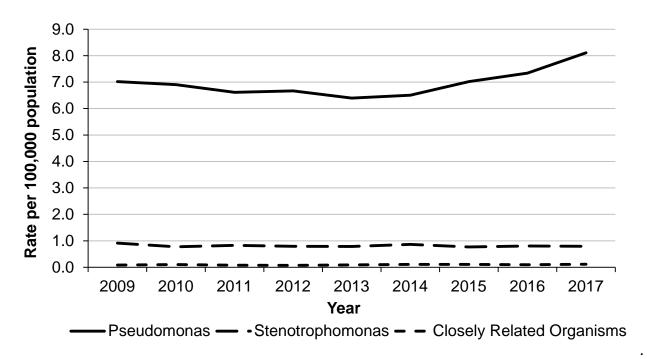
Figure 1 shows the trend in the rate (per 100,000 population) of laboratory-reported bacteraemia in England, Wales and Northern Ireland between 2009 and 2017, due to *Pseudomonas* spp., *Stenotrophomonas* spp. and closely related genera (*Brevudimonas*, *Burkholderia*, *Comamonas*, *Ralstonia and Shewanella*).

There was an overall 15.7% increase in the rate of *Pseudomonas* spp. bacteraemia (from 7.0 to 8.1 reports per 100,000 population) between 2009 and 2017. However, year-to-year fluctuation was seen during this period with the rate decreasing between 2009 and 2013, but increasing year-on-year between 2013 and 2017, with a 11.0% increase between 2016 and 2017 alone (from 7.3 to 8.1 reports per 100,000 population) (figure 1).

There was an overall 11.1% decrease in the rate of *Stenotrophomonas* bacteraemia between 2009 and 2017 (from 0.9 to 0.8 per 100,000 population).

Between 2009 and 2016, the rate of bacteraemia due to other genera closely related to *Pseudomonas* species increased by 11.1% from 0.09 to 0.1 reports per 100,000 population.

Figure 1. *Pseudomonas* spp., *Stenotrophomonas* spp. and related genera bacteraemia rate per 100,000 population (England Wales and Northern Ireland): 2009 to 2017



Geographic distribution: Pseudomonas

The combined rate of bacteraemia due to *Pseudomonas* spp. in England, Wales and Northern Ireland was 8.1 per 100,000 population in 2017; individually, the rates were 5.6 per 100,000 in Northern Ireland, 7.6 per 100,000 population in Wales and 8.2 per 100,000 population in England. The rate varied in England across PHE centres (PHECs) from 6.4 per 100,000 population in North West to 9.2 per 100,000 in London (figure 2a) (table 1a).

The rate in England has increased each year from 2013, while the rates of *Pseudomonas* spp. bacteraemia in Northern Ireland and Wales have fluctuated overall. Increases of 28.1%, 16.7% and 2.7% were observed between 2013 and 2017 in England, Northern Ireland and Wales, respectively (table 1a). The bacteraemia rates were similar in Wales and Northern Ireland between 2016 and 2017 with 7.4-7.6 and 5.6-5.7 reports per 100,000 population respectively. An increase of 11.2% was seen in England (7.4 to 8.2 reports per 100,000) between 2016 and 2017.

There was an overall increase in rates of *Pseudomonas* spp. bacteraemia observed for England between 2013 and 2017. Between 2016 and 2017, the greatest increases in bacteraemia rates seen were in the West Midlands and East Midlands PHE Centres, rates increased by 21.4% (7.0 to 8.5 reports per 100,000 population) and 20.0% (7.0 to 8.4 reports per 100,000 population), respectively. The bacteraemia rate nominally decreased by 5.1% (from 5.9 to 5.6 reports per 100,000 population) in Northern Ireland between 2015 and 2017. The bacteraemia rate in Wales has increased by 13.4% (6.7 to 7.6 per 100,000 population) between 2014 and 2017.

Between 2013 and 2017, the highest increase in *Pseudomonas* spp. bacteraemia rates of 51.9% (5.4 to 8.2 reports per 100,000 population) and 35.8% (6.7 to 9.1 reports per 100,000 population) were observed in North East and South East of England PHECs, respectively. The rest of the Centres reported increases in their *Pseudomonas* spp. bacteraemia rates between 2016 and 2017. Northern Ireland reported a decrease in their rates between 2016 and 2017(1.8%; 5.7 to 5.6 reports per 100,000 population).

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Figure 2a. Geographical distribution of *Pseudomonas* spp. bacteraemia rates per 100,000 population (England, Wales and Northern Ireland): 2017

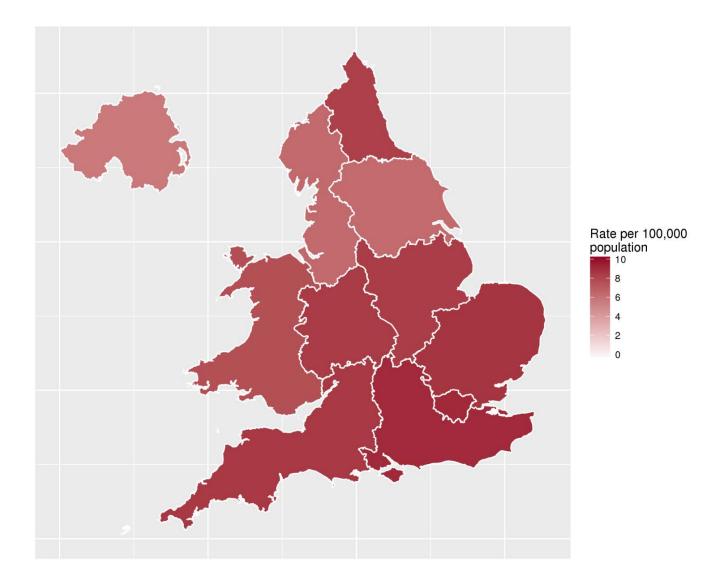


Table 1a. Rate of *Pseudomonas* spp. bacteraemia reports per 100,000 population by PHE Centre (England, Wales and Northern Ireland): 2013 to 2017

		R	ate per 1	00,000 p	opulatio	n
Region	PHE Centre	2013	2014	2015	2016	2017
North of	North East	5.4	5.4	6.1	6.9	8.2
England	Yorkshire and Humber	5.0	4.7	6.2	6.0	6.3
	North West	5.9	5.5	5.9	6.1	6.4
Midlands	West Midlands	6.9	7.1	7.7	7.0	8.5
and East of	East Midlands	6.1	6.3	6.7	7.0	8.4
England	East of England	7.0	6.8	8.1	8.2	8.7
London	London	7.1	8.2	7.8	8.0	9.2
South of	South West	6.3	6.6	6.8	8.4	8.6
England	South East	6.7	6.7	7.4	8.1	9.1
England*		6.4	6.5	7.1	7.4	8.2
Northern Irela	and [†]	4.8	5.3	5.9	5.7	5.6
Wales*		7.4	6.7	6.8	7.4	7.6
England, Wa	ales & Northern Ireland	6.4	6.5	7.0	7.3	8.1

^{*} Extracted on 17 May 2018; † Extracted on 15 June 2018, * Extracted on 12 February 2018

Geographic distribution: Stenotrophomonas

The combined rate of bacteraemia due to *Stenotrophomonas* spp. in England, Wales and Northern Ireland was 0.8 (n=477) reports per 100,000 population in 2017; individually the rates of laboratory-reported bacteraemia were 1.3 per 100,000 population in Wales, 1.0 per 100,000 population in Northern Ireland and 0.8 per 100,000 population in England in 2017. The rate varied in England across PHECs between 0.5 bacteraemia reports per 100,000 population in West Midlands PHEC to 1.0 bacteraemia reports per 100,000 population in North East PHEC (figure 2b) (table 1b).

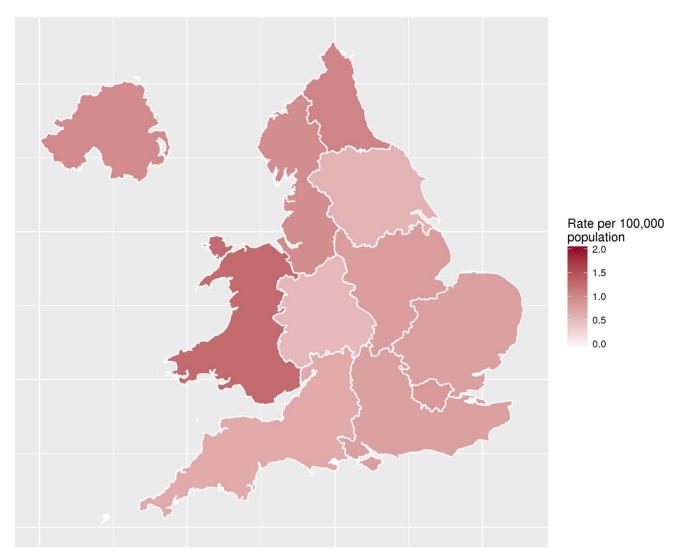
Between 2013 and 2017, the highest increase in the bacteraemia rate was observed in Wales (85.7%; 0.7 to 1.3 reports per 100,000 population), albeit the rate peeked slightly higher in 2015 with a rate of 1.4 bacteraemia reports per 100,000 population. The bacteraemia rate in Northern Ireland decreased by 33.3% (1.5 to 1.0 reports per 100,000 population) between 2013 and 2017 while over the same time period the rate remained unchanged at 0.8 reports per 100,000 population in England.

The *Stenotrophomonas* bacteraemia rate increase was observed among five PHECs, of which the highest were in East of England (33.3%; 0.6 to 0.8 reports per 100,000 population) and Yorkshire and Humber (20.0%; 0.5 to 0.6 reports per 100,000 population)

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PHECs, with the rest of the Centres reporting decreases in their rates between 2013 and 2017. During this five-year period, the greatest reduction in rates (28.6%) was observed in the West Midlands PHEC (0.7 to 0.5 reports per 100,000); however, between 2015 and 2016, they also experienced the highest increase in rate (60.0%) from 0.5 to 0.8 reports per 100,000 population. The greatest reported reduction between 2016 and 2017 (28.6%) was observed in the West Midlands PHEC (0.7 to 0.5 reports per 100,000).

Figure 2b. Geographical distribution of *Stenotrophomonas* spp. bacteraemia rates per 100,000 population (England, Wales and Northern Ireland): 2017



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Table 1b. Rate of *Strenotrophomonas* spp. bacteraemia reports per 100,000 population by PHE Centre (England, Wales and Northern Ireland): 2013 to 2017

		R	ate per 1	00,000 p	opulatior)
Region	PHE Centre	2013	2014	2015	2016	2017
North of	North East	0.9	1.0	0.8	0.8	1.0
England	Yorkshire and Humber	0.5	0.6	0.6	0.6	0.6
	North West	1.1	1.1	0.9	0.8	0.9
Midlands	West Midlands	0.7	0.6	0.5	0.8	0.5
and East	East Midlands	0.7	0.8	0.5	0.8	8.0
of England	East of England	0.6	0.7	0.8	0.7	0.8
London	London	0.9	1.3	0.9	0.9	0.8
South of	South West	0.6	0.7	0.6	0.8	0.7
England	South East	0.7	0.7	0.7	0.7	0.8
England*		8.0	0.9	0.7	0.8	8.0
Northern Ire	land [†]	1.5	1.1	1.2	1.2	1.0
Wales [¥]		0.7	0.9	1.4	1.3	1.3
England, W	ales & Northern Ireland	8.0	0.9	8.0	8.0	8.0

^{*} Extracted on 17 May 2018; † Extracted on 15 June 2018, * Extracted on 12 February 2018

It is of note that, in England and Northern Ireland, there are links from the different laboratories to SGSS/CoSurv that report clinically significant isolates. In Wales, data are collected by extraction from a single laboratory information system used by all the microbiology laboratories. The system extracts all positive blood cultures, including those not thought to be clinically significant.

Species distribution

In 2017, 91% (n=4,431/4,883) of *Pseudomonas* isolates from blood in England, Wales and Northern Ireland were identified to species level, however this is impacted by the limitations of MALDI-ToF analysis, which does not reliably distinguish between closely related species such as those in the *P. fluorescens* or *P. putida* groups (table 2a). The proportion identified to species level was broadly similar to previous years. *P. aeruginosa* was the most commonly isolated species in 2017 as in previous years, accounting for approximately 82% of all isolates of this genus. There had been a slight downward trend in these figures, with *P. aeruginosa* accounting for 83%, 82%, 81% and 80% of all *Pseudomonas* species bacteraemias in 2013, 2014, 2015 and 2016, respectively. *P. aeruginosa* is the third most common cause of Gram-negative bacteraemia and, along with *Escherichia coli* and *Klebsiella* spp., is one of the key pathogens on which the government is focusing its ambition to halve healthcare-associated Gram-negative blood stream infections by financial year 2020/21 [2].

In 2017, the most commonly isolated *Stenotrophomonas* species was *Stenotrophomonas maltophilia* at 98% (n=469/477) in England, Wales and Northern Ireland (table 2b). This is broadly similar to *Stenotrophomonas* species bacteraemia reports in other years with the exception of 2014, when the proportion was 92% (n=470/513) due to a large number of isolates not having species level information recorded. To date, *S. maltophilia* is the only known opportunistic human pathogen in the *Stenotrophomonas* genus, which makes it likely that the small number of isolates without species data is in fact *S. maltophilia* [3].

Burkholderia spp. and *Brevudimonas* spp. were the most commonly reported related organisms* between 2013 and 2017. In 2017, these accounted for 42% (n=49/116) and 36% (n=42/116) of all closely related organisms, respectively (table 2c). There was a small overall decline in the number of reports of related organisms between 2016 and 2017.

^{*} Related organisms include genera where at least one species has previously been classified as *Pseudomonas* spp. or *Stenotrophomonas* spp.

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Table 2a. Reports of Pseudomonas spp. bacteraemia by species (England, Wales and Northern Ireland): 2013 to 2017

	2013		2014		2015		2016		2017	
	No.	%								
Pseudomonas spp.	3,759	100	3,853	100	4,191	100	4,422	100	4,883	100
P. aeruginosa	3,111	83	3,144	82	3,412	81	3,556	80	4,019	82
P. alcaligenes	2	<1	4	<1	7	<1	8	<1	5	<1
P. chlororaphis	0	0	0	0	1	<1	1	<1	0	0
P. citronellolis	0	0	0	0	0	0	0	0	1	<1
P. fluorescens group*	54	1	56	1	37	1	44	<1	31	1
P. koreensis	0	0	1	<1	2	<1	1	<1	7	<1
P. libanensis	0	0	0	0	0	0	0	0	1	<1
P. luteola	2	<1	2	<1	4	<1	8	<1	10	<1
P. mendocina	1	<1	7	<1	5	<1	6	<1	16	<1
P. oleovorans	0	0	0	0	1	<1	5	<1	1	<1
P. otitidis	0	0	1	<1	0	0	0	0	0	0
P. paucimobilis	65	2	63	2	56	1	65	1	62	1
P. putida group**	61	2	85	2	100	2	140	3	166	3
P. stutzeri	83	2	94	2	92	2	84	2	81	2
P. thomasii	1	<1	0	0	1	<1	1	<1	0	0
Pseudomonas spp.,										
other named	72	2	78	2	45	1	35	1	31	1
Pseudomonas spp.,										
sp. not recorded	307	8	318	8	428	10	468	11	452	9

^{*} P. fluorescens and P. tolaasii

^{**} P. putida, P. fulva, P. monteilii, P. mosselii and P. oryzihabitans

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Table 2b. Reports of Stenotrophomonas spp. bacteraemia by species (England, Wales and Northern Ireland): 2013 to 2017

	2013		2014		2015		2016		2017	
	No.	%								
Stenotrophomonas spp.	460	100	513	100	460	100	483	100	477	100
S. acidaminiphila	0	0	0	0	0	0	0	0	3	1
S. maltophilia	448	97	470	92	453	98	479	99	469	98
Stenotrophomonas spp., species										
not recorded	12	3	43	8	7	2	4	1	5	1

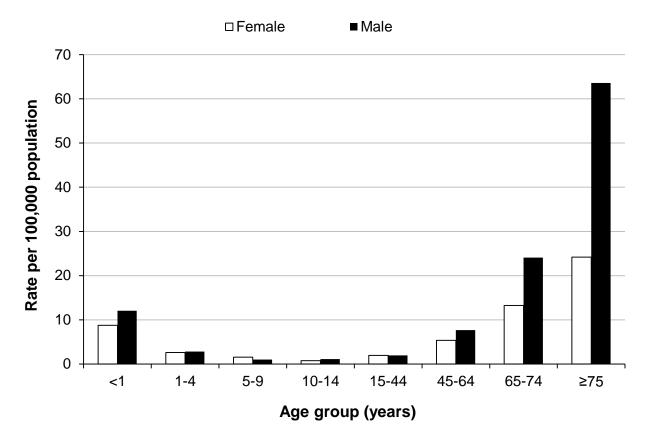
Table 2c. Reports of related organisms bacteraemia by genus (England, Wales and Northern Ireland): 2013 to 2017

	2013		2014		2015		2016		2017	
	No.	%								
Related Organisms	101	100	94	100	127	100	122	100	116	100
Brevundimonas spp.	32	32	40	43	42	33	40	33	42	36
Burkholderia spp.	50	50	31	33	66	52	65	53	49	42
Comamonas spp.	7	7	12	13	12	9	11	9	9	8
Ralstonia spp.	8	8	8	9	4	3	4	3	10	9
Shewanella spp.	4	4	3	3	3	2	2	2	6	5

Age and sex distribution

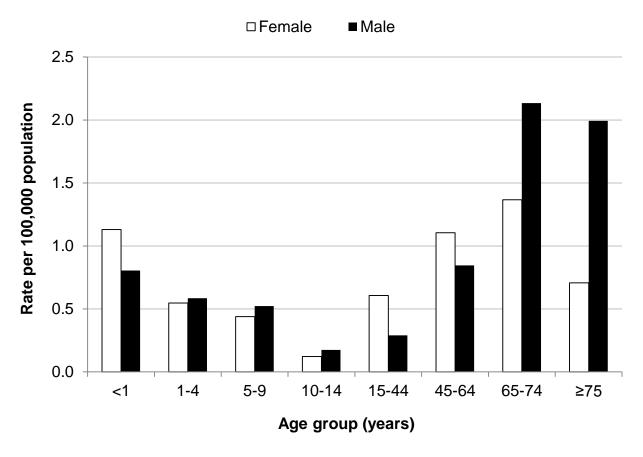
Figure 3a depicts *Pseudomonas* bacteraemia rate per 100,000 population amongst men and women across different age groups in England, Wales and Northern Ireland in 2017. The bacteraemia rate was the highest among older age groups (≥75 years and 65 to 74 years of age) and those below the age of one year. The rate of bacteraemia per 100,000 population in these age groups was markedly higher amongst males in comparison to females (≥75 years: 63.6 vs. 24.2, 65-74 years: 24.1 vs. 13.2, and <1 year: 12.1 vs. 8.8 per 100,000 population). Among 5-9 year-olds, the rate was observed to be higher in females than males (1.5 vs. 1.0 per 100,000 population) in 2017.

Figure 3a. *Pseudomonas* bacteraemia rates per 100,000 population by age and sex (England, Wales and Northern Ireland): 2017



The rate of S*tenotrophomonas* bacteraemia in males was highest amongst those aged 75 years and older, 65-74 years old and 45-64 year olds (2.0, 2.1 and 0.8 per 100,000 population). The rate in females was the highest amongst 65-74 year olds, 45-64 year olds and those below the age of one (1.4, 1.1 and 1.1 per 100,000 population, respectively). Generally, the rate was higher amongst men in comparison to women with the exception of 45-64 year olds, 15-44 year olds, and those below the age of one (1.1 vs. 0.8, 0.6 vs. 0.3 and 1.1 vs 0.8 per 100,000 population in women and men, respectively).

Figure 3b. *Stenotrophomonas* bacteraemia rates per 100,000 population by age and sex (England, Wales and Northern Ireland): 2017



Antimicrobial resistance: England and Northern Ireland

The percentage of *Pseudomonas aeruginosa* and *Stenotrophomonas* bacteraemia isolates with susceptibility test results reported in 2017 ranged from 86-97% and 93%, respectively, for key antimicrobials.

Susceptibility results reported for key antimicrobials for *P. aeruginosa* are presented in table 3a. Between 2015 and 2016, the resistance patterns for key antimicrobial agents remained broadly stable with small decreases in resistance for gentamicin (4% to 3%) and tobramycin (from 4% to 3%). Increases in resistance to imipenem (9% to 11%), amikacin (1% to 2%) and piperacillin\tazobactam (6% to 7%) were observed over the same time period. Resistance to ceftazidime and meropenem remained stable between 2015 and 2016, at 6% and 5%, respectively. The highest percentage of resistance was observed for the following in 2017: imipenem (11%), ciprofloxacin (8%), ceftazidime (7%), piperacillin\tazobactam (7%) and meropenem (6%) (table 3a). These results are in line with P. aeruginosa being resistant to a multitude of antibiotics through intrinsic and adaptive mechanisms [4]. The resistance patterns to imipemen, meropenem and ciprofloxacin presented in this report indicate the pathogen's ability to develop antibiotic resistance through three main mechanisms - alteration in DNA gyrase by mutation in gyrA or gyrB genes, decreased drug accumulation by decreased permeability of the cell wall and enhanced efflux [5]. The loss of OprD in P. aeruginosa cells affects carbapenem uptake, underlying the non-susceptibility to imipenem and meropenem [4,6]. Mutations in gyrA and gyrB and parC and parE reducing binding affinity of fluoroquinolones are believed to be involved in resistance to ciprofloxacin [4,5].

Antimicrobial resistance rates have overall remained stable for *P. aeruginosa* between 2016 and 2017, however, a 1% increase in resistance was seen for the following during this period: gentamicin (3% to 4%), ciprofloxacin (7% to 8%), ceftazidime (6% to 7%) and meropenem (from 5% to 6%).

The resistance of *P. aeruginosa* in blood samples was shown to be lower than in respiratory isolates for all antimicrobials tested, according to the British Society for Antimicrobial Chemotherapy Resistance Surveillance Project [7].

Pseudomonas species isolates exhibiting increased drug resistance are of particular concern for patients with weakened immune systems. This has resulted in the inclusion of

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Pseudomonas as one of the key groups of pathogens to monitor as part of the UK fiveyear Antimicrobial Resistance Strategy and the English Surveillance Programme for Antimicrobial Utilisation and Resistance [8,9].

The proportion of reduced susceptibility (intermediate) to Piperacillin\Tazobactam remained the same between 2015 and 2016, but increased between 2016 and 2017 from 3% to 7%. This is suspected to be due to an artefact associated with automated antimicrobial susceptibility testing. Reduced susceptibility of other antimicrobial agents has remained broadly similar between 2015 and 2017.

Antibiotic susceptibility results for co-trimoxazole in *Stenotrophomonas* isolates are presented in table 3b. This antimicrobial was selected because it is the drug of choice for treatment of *S. maltophilia* [10,11]. For *Stenotrophomonas* bacteraemia, an increase in resistance to co-trimaxazole was observed between 2016 and 2017 (6% to 7%).

Table 4 presents analysis of resistance of *Pseudomonas* species (including *P. aeruginosa*) isolates resistant to more than one antibiotic agent in England in 2017. Overall, the percentage of resistance reported for the different antimicrobial combinations (gentamicin, ciprofloxacin and ceftazidime) were similar, and multi-drug resistance is rare (≤ 2%) in the listed combinations.

For advice on treatment of antibiotic-resistant infections due to these organisms or for reference services including species identification and confirmation of susceptibility testing results, laboratories should contact PHE's AMRHAI Reference Unit in London [12].

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Table 3a. Antibiotic susceptibility for *Pseudomonas aeruginosa* bacteraemia in England, Wales and Northern Ireland: 2015 to 2017

2015					2016		2017			
Antimicrobial agent	S* (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	
Gentamicin	96	<1	4	96	<1	3	95	<1	4	
Ciprofloxacin	90	2	7	90	2	7	89	2	8	
Ceftazidime	94	<1	6	93	<1	6	93	<1	7	
Meropenem	92	2	5	92	3	5	90	4	6	
Imipenem	88	3	9	87	2	11	86	3	11	
Tobramycin	96	<1	4	97	<1	3	97	0	3	
Amikacin	97	1	1	97	1	2	96	2	2	
Piperacillin\Tazobactam	91	3	6	90	3	7	86	7	7	

^{*}S = susceptible; I = intermediate (reduced susceptibility); R = resistant

Table 3b. Antibiotic susceptibility for Stenotrophomonas bacteraemia in England, Wales and Northern Ireland: 2015 to 2017

		2015			2016			2017	
Antimicrobial agent	S* (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Co-trimoxazole	94	0	6	94	0	6	93	0	7

^{*}S = susceptible; I = intermediate (reduced susceptibility); R = resistant

Table 4. Multi-drug antimicrobial testing and resistance summary for *Pseudomonas* bacteraemia (England): 2015 to 2017

		2015			20	16	2017		
Antimicrobial combinations	S*	I	R	S	I	R	S	I	R
/	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Gentamicin and Ciprofloxacin	98	<1	2	98	<1	2	98	<1	2
Gentamicin and Ceftazidime	99	<1	1	99	<1	1	98	<1	2
Ciprofloxacin and Ceftazidime	98	<1	2	98	<1	2	97	<1	2
Gentamicin, Ciprofloxacin	99	<1	1	99	<1	1	99	<1	1
and Ceftazidime									

^{*}S = susceptible; I = intermediate (reduced susceptibility); R = resistant

Acknowledgements

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Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. We do this through world-class science, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health, and are a distinct delivery organisation with operational autonomy to advise and support government, local authorities and the NHS in a professionally independent manner.

About Health Protection Report

Health Protection Report is a national public health bulletin for England and Wales, published by Public Health England. It is PHE's principal channel for the dissemination of laboratory data relating to pathogens and infections/communicable diseases of public health significance and of reports on outbreaks, incidents and ongoing investigations.

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