Antimicrobial resistance and molecular epidemiology of *Neisseria gonorrhoeae* in New Zealand, 2014-15

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SUMMARY

In recent years there have been reports from many parts of the world of *Neisseria gonorrhoeae* strains displaying decreased susceptibility to third-generation cephalosporins. Dual therapy with the third-generation cephalosporin, ceftriaxone, and azithromycin is the current standard therapy for gonorrhoea in New Zealand (NZ). *N. gonorrhoeae* with decreased susceptibility to ceftriaxone have been identified in the Auckland area, and multi-antigen sequence typing (NG-MAST) of these isolates has shown that some belong to international clones that are associated with decreased ceftriaxone susceptibility, including ST1407. This national survey was undertaken to (1) provide information on the antimicrobial susceptibility of *N. gonorrhoeae* isolated throughout NZ, including susceptibility to antimicrobials that have been proposed as alternatives to ceftriaxone, such as spectinomycin, gentamicin and ertapenem; and (2) determine NG-MAST types among *N. gonorrhoeae* and any associations between types and susceptibility patterns.

N. gonorrhoeae isolates were collected from laboratories throughout NZ between October 2014 and May 2015. Susceptibility to azithromycin, cefixime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, penicillin, spectinomycin and tetracycline was determined by agar dilution according to the methods of the Clinical and Laboratory Standards Institute (CLSI). Ciprofloxacin, penicillin, spectinomycin and tetracycline minimum inhibitory concentrations (MICs) were interpreted according to CLSI breakpoints. Azithromycin MICs were interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints. β-lactamase production was determined using the chromogenic cephalosporin nitrocefin. The NG-MAST types were derived *in silico* from assembled Illumina short-read data.

A total of 425 non-duplicate isolates were included in the survey. Over two-thirds (69.4%) of the isolates came from laboratories serving the greater Auckland area. Eleven (2.6%) isolates had decreased ceftriaxone susceptibility (MIC ≥0.06 mg/L) and four (0.9%) had decreased cefixime susceptibility (MIC ≥0.12 mg/L). Of the 11 isolates with decreased ceftriaxone susceptibility, 8 were isolated from patients in the greater Auckland area. The rates of resistance to other antibiotics were: azithromycin, 1.7%, with a further 9.4% intermediate resistance; ciprofloxacin, 32.2%; penicillin, 12.0%; spectinomycin, 0.0%; and tetracycline, 26.1%. There are no interpretive standards for ertapenem and gentamicin, but the MIC_{50} and MIC_{90} values for ertapenem were 0.016 and 0.03 mg/L, respectively, and for

gentamicin both the MIC_{50} and MIC_{90} values were 8 mg/L. One isolate with decreased ceftriaxone susceptibility was also azithromycin resistant.

A diverse range of NG-MAST types were identified. The most prevalent NG-MAST types were ST4186, which accounted for 45 (11.3%) of the 399 typed isolates, and ST2400, which accounted for 43 (10.8%) of the isolates. Sixty-three (53.8%) of the total 117 NG-MAST types identified were associated with just one isolate and another 23 (19.7%) types were associated with just two isolates. The NG-MAST types identified among the isolates with decreased susceptibility to ceftriaxone and cefixime were variable, and none were ST1407. In contrast three of the seven azithromycin-resistant isolates were ST10193 and these three isolates were from Canterbury DHB patients.

Rates of antimicrobial resistance among gonococci in NZ are generally comparable to other developed countries. Reassuringly, the prevalence of decreased susceptibility to ceftriaxone found in this survey was low and none of the isolates with decreased susceptibility belonged to the globally disseminated ST1407 clone. However, of concern was the finding that 11.1% of isolates were non-susceptible to azithromycin. Unfortunately, due to the limited amount of culturing for *N. gonorrhoeae* now undertaken in many NZ laboratories as a result of the trend to nucleic acid-based diagnosis of gonorrhoea, gonococci circulating in regions outside the greater Auckland area were under-represented in this 'national' survey. This finding highlights the need for laboratories to maintain a level of gonococcal culture sufficient to monitor resistance patterns.

1. INTRODUCTION

Antimicrobial resistance in *Neisseria gonorrhoeae* has been identified as a major global public health threat. Moreover, the trend to nucleic acid-based diagnosis of gonorrhoea has resulted in fewer isolates of *N. gonorrhoeae* being available for antimicrobial susceptibility testing and molecular characterisation.[1] Data from the national surveillance of sexually transmitted infections (STIs) suggests there have been recent increases in gonorrhoea in at least some parts of New Zealand.[2] For several years, diagnostic laboratories have submitted their gonococcal antimicrobial susceptibility data to the Institute of Environmental Science and Research (ESR) on an annual basis. This surveillance covered β-lactamase production, penicillin resistance, ciprofloxacin resistance and tetracycline resistance among gonococci, and data was collated to produce aggregate national estimates of resistance (see https://surv.esr.cri.nz/antimicrobial/general_antimicrobial_susceptibility.php). Data on ceftriaxone susceptibility in gonococci was not collected as part of this surveillance system, although, since January 2013, limited ceftriaxone susceptibility data has been collected as part of ESR's laboratory-based STI surveillance system.[2]

Isolates of *N. gonorrhoeae* with decreased susceptibility to ceftriaxone have previously been identified in the Auckland area, and molecular typing data shows that some of these isolates are associated with distinct overseas clones of *N. gonorrhoeae*, including NG-MAST (*N. gonorrhoeae* multi-antigen sequence typing) ST1407.[3] To date, there have been few systematic surveys providing data on the antimicrobial susceptibility and molecular types of *N. gonorrhoeae* in New Zealand. Accordingly, to provide contemporary information on antimicrobial resistance patterns and the molecular epidemiology of *N. gonorrhoeae* in New Zealand, ESR analysed *N. gonorrhoeae* isolates collected from laboratories throughout New Zealand between October 2014 and May 2015. Specifically, the aims of this survey were to:

- provide information on antimicrobial resistance among *N. gonorrhoeae*, including resistance to antimicrobials that have been proposed as alternatives to ceftriaxone, such as spectinomycin, gentamicin and ertapenem; and
- determine NG-MAST types among *N. gonorrhoeae* and any associations between types and susceptibility patterns.

2. METHODS

2.1 ISOLATES AND PATIENT INFORMATION

Laboratories were requested to refer all *N. gonorrhoeae* isolates cultured between 27 October 2014 and 29 May 2015 to ESR for the survey. Only those laboratories that provide the majority of the local diagnostic services for STI specimens were requested to participate. These laboratories are listed in Table 4 in the Appendix. Because of the limited number of specimens routinely cultured for *N. gonorrhoeae* in several laboratories, some laboratories undertook to collect specimens for culture from an increased proportion of patients for the duration of the survey. This enhanced rate of culture was usually applied to specimens from patients attending sexual health clinics only.

When referring isolates for the survey, diagnostic laboratories supplied epidemiological data including the patient's NHI number or another identifier, the patient's sex and date of birth, and the source or site of the specimen from which *N. gonorrhoeae* was cultured. The patient's domicile and therefore district health board (DHB) was inferred from the location of the referring laboratory unless the referring laboratory specified otherwise. For the purposes of DHB allocation, the three DHBs (Waitemata, Auckland and Counties Manukau) in the greater Auckland area were grouped together as the patient's specific DHB could not be inferred on the basis of the referring laboratory. For similar reasons, the two DHBs (Capital & Coast and Hutt Valley) in the greater Wellington area were also grouped together.

2.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility to azithromycin, cefixime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, penicillin, spectinomycin and tetracycline was determined by agar dilution according to the methods of the Clinical and Laboratory Standards Institute (CLSI).[4] Ertapenem was purchased from TokuE (Bellingham, Washington, United States). All other antibiotic pure substances used for agar dilution were purchased from Sigma-Aldrich (Saint Louis, Missouri, United States).

β-lactamase production was determined using the chromogenic cephalosporin nitrocefin.

Ciprofloxacin, penicillin, spectinomycin and tetracycline minimum inhibitory concentrations (MICs) were interpreted according to CLSI breakpoints.[5] Azithromycin MICs were interpreted according to the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints.[6] Decreased susceptibility to ceftriaxone and cefixime was defined as an

MIC ≥0.06 and ≥0.12 mg/L, respectively. No interpretive standards are available for ertapenem or gentamicin.

The MIC_{50} and MIC_{90} values were defined as the MICs at which at least 50% and 90%, respectively, of isolates were inhibited.

2.3 NG-MAST TYPING

The sequences for the *por* and *tbpB* loci were derived *in silico*, from assembled Illumina short-read data. Allele numbers for both loci and the NG-MAST types were assigned on the basis of data in the NG-MAST database (http://www.ng-mast.net). Where the *por* or *tbpB* allele was novel (ie, not found in the NG-MAST database), or the combination of *por* and *tbpB* alleles was novel, temporary allele numbers and/or NG-MAST types were assigned. In this report, these temporary NG-MAST types commence with '900' and have the number format '900nnn'.

A subset of 401 isolates was selected for typing by randomly excluding 24 isolates referred by LabPlus and Labtests.

2.4 DATA ANALYSIS

Geographic analyses were by district health board (DHB) and by the Northern, Midland, Central and Southern regions. The Northern region includes Northland, Waitemata, Auckland and Counties Manukau DHBs; the Midland region includes Waikato, Lakes, Bay of Plenty, Tairawhiti and Taranaki DHBs; the Central region includes Hawke's Bay, Whanganui, MidCentral, Hutt, Capital and Coast, Wairarapa and Nelson Marlborough DHBs; and the Southern region includes West Coast, Canterbury, South Canterbury and Southern DHBs.

Statistical analyses were performed with SAS software v.9.3 (SAS Institute Inc, Cary, North Carolina, United States).

3. RESULTS

3.1 ISOLATES AND PATIENT DEMOGRAPHICS

A total of 425 non-duplicate *N. gonorrhoeae* isolates were received for the survey. The laboratories that referred isolates, and the number of isolates each laboratory referred, are presented in Table 4 in the Appendix. Over two-thirds (69.4%, 295/425) of the isolates came from laboratories serving the greater Auckland area.

The age and sex distribution of the patients from whom *N. gonorrhoeae* were isolated and referred for the survey is shown in Table 1. 80.9% (344) of the total 425 isolates were from male patients. The majority of patients were between 15 and 34 years of age.

Table 1. Age and sex of patients from whom *Neisseria gonorrhoeae* were referred

Age group (years)	Number (column percent)[1]:					
	Female		Male		Total	
0-4	1	(1.3)	0	(-)	1	(0.3)
5-9	0	(-)	0	(-)	0	(-)
10-14	3	(3.9)	1	(0.3)	4	(1.0)
15-19	21	(27.3)	47	(14.6)	68	(17.1)
20-24	24	(31.2)	81	(25.2)	105	(26.4)
25-29	12	(15.6)	67	(20.9)	79	(19.9)
30-34	7	(9.1)	46	(14.3)	53	(13.3)
35-39	3	(3.9)	28	(8.7)	31	(7.8)
≥40	6	(7.8)	51	(15.9)	57	(14.3)
Total[1]	81		344		425	

1 Age not reported for 27 patients: 4 females and 23 males. These patients are included in the totals for each gender, but not in the denominator used to calculate the percentages for each age group.

The sites among female and male patients, from which the specimen that yielded *N. gonorrhoeae* was taken, are presented in Table 5 in the Appendix. Among males, 66.2% of the isolates were cultured from urethral specimens, 16.0% from anorectal specimens, and 12.8% from penile sites.

The geographic distribution of the patients, from whom the *N. gonorrhoeae* isolates included in the survey were isolated, is presented in Table 6 in the Appendix. This data shows that the isolates received for the survey are likely to underrepresent gonococci circulating in the

Midland, Central and Southern regions. While 62% of the population resides in these three regions, only 28.5% of the isolates included in the survey came from these regions with the remainder (71.5%) referred from the Northern region.

3.2 ANTIMICROBIAL SUSCEPTIBILITY

The antimicrobial susceptibility of the 425 isolates included in the survey is shown in Table 2. The full MIC distribution data for each antimicrobial tested is presented in Table 7 in the Appendix.

Table 2. Antimicrobial susceptibility among *Neisseria gonorrhoeae*, 2014-2015

Antimicrobial	Percent (number):			mg/L	
	Susceptible	Intermediate/ decreased susceptibility[1]	Resistant	MIC_{50}	MIC_{90}
penicillin	1.9 (8)	86.1 (366)	12.0 (51)	0.5	2
cefixime	99.1 (421)	0.9 (4)	0.0 (0)	0.008	0.03
ceftriaxone	97.4 (414)	2.6 (11)	0.0 (0)	0.004	0.03
ertapenem[2]	-	-	-	0.016	0.03
ciprofloxacin	67.8 (288)	0.0 (0)	32.2 (137)	0.004	≥8
tetracycline	32.0 (136)	41.9 (178)	26.1 (111)	0.5	16
spectinomycin	98.6 (419)	1.4 (6)	0.0 (0)	32	32
azithromycin	88.9 (378)	9.4 (40)	1.7 (7)	0.25	0.5
gentamicin[2]	-	-	-	8	8

1 The 'decreased susceptibility' category applies to cefixime and ceftriaxone.
2 No interpretive standards available for ertapenem and gentamicin.

Eleven (2.6%) of the 425 isolates were categorised as having decreased ceftriaxone susceptibility (MIC ≥0.06 mg/L) and four (0.9%) as having decreased cefixime susceptibility (MIC ≥0.12 mg/L). None of the 11 isolates with decreased ceftriaxone susceptibility had decreased cefixime susceptibility. One of the 11 isolates with decreased ceftriaxone susceptibility was also azithromycin resistant, which equates to a dual decreased ceftriaxone susceptibility and azithromycin resistance prevalence of just 0.2% (1/425).

Of the 11 isolates with decreased ceftriaxone susceptibility, 8 were isolated from patients in one of the three DHBs in the Auckland area, 2 were from patients in one of the two DHBs in the Wellington area, and the remaining isolate was from a Taranaki DHB patient.

While only 1.7% (7) of isolates were azithromycin resistant, a further 9.4% (40) of isolates had intermediate resistance, making a total of 11.1% azithromycin non-susceptibility. Among the 12.0% (51) of isolates that were penicillin resistant, 4.9% (21) produced β-lactamase and presumably the other 7.1% (30) had chromosomally mediated penicillin resistance. Among the 26.1% (111) of isolates that were tetracycline resistant, 15.8% (67) had MICs ≥16 mg/L indicating these isolates have high-level, plasmid-mediated tetracycline resistance due to the *tetM* gene.

3.3 NG-MAST TYPES

The NG-MAST type was able to be determined for 399 of the 401 isolates typed, and 117 distinct NG-MAST types were identified among these 399 isolates (Table 8 in the Appendix). Sixty-six (56.4%) of these types were novel, that is, not currently identified in the NG-MAST database at http://www.ng-mast.net.

The most prevalent NG-MAST types were ST4186, which accounted for 45 (11.3%) of the 399 typed isolates, and ST2400, which accounted for 43 (10.8%) of the isolates (Table 8 in the Appendix). Sixty-three (53.8%) of the total 117 NG-MAST types identified were associated with just one isolate, with another 23 (19.7%) types associated with just two isolates.

The NG-MAST types identified among the isolates with decreased susceptibility to ceftriaxone and cefixime were variable. Among the 10 isolates with decreased ceftriaxone susceptibility that were typed, eight types were identified: ST2400 (2 isolates), ST3356 (1), ST8883 (2), ST8901 (1), ST9368 (1), ST90007 (1), ST 90019 (1) and ST90052 (1). Each of the four isolates with decreased cefixime susceptibility was a different type: ST4186, ST5595, ST9368 and ST90024.

Among the azithromycin non-susceptible isolates there was some association with NG-MAST type ST10193. Three of the seven azithromycin-resistant (MICs 1 mg/L) isolates were ST10193 and these three isolates were from Canterbury DHB patients. A further 10 of the 36 azithromycin-intermediate (MICs 0.5 mg/L) isolates that were typed were also ST10193, with 5 of these 10 isolates from Canterbury DHB patients.

4. DISCUSSION

Over the past decade, there have been increasing reports of emerging antimicrobial resistance in *N. gonorrhoeae,* particularly to extended-spectrum cephalosporins such as ceftriaxone, which currently forms the mainstay of treatment. Successful public health control of gonorrhoea relies on effective antimicrobial therapy, which, in turn, is informed by knowledge of local resistance patterns. As part of the Global Action Plan to control the spread of antimicrobial resistance in *N. gonorrhoeae,* the World Health Organization has called for enhanced surveillance activities.[7] In this context, we assessed the antimicrobial resistance patterns and molecular epidemiology of *N. gonorrhoeae* in New Zealand over a 7-month period.

Overall, rates of antimicrobial resistance were generally comparable to other developed countries. For example, Table 3 compares results from this survey with the most recent data (2014) from the Australian Gonococcal Surveillance Programme (AGSP), which has continuously monitored resistance in *N. gonorrhoeae* in Australia since 1981.[8] In general, rates of resistance in New Zealand were similar, or lower, although it is important to note that differences in the tested populations may partially contribute to differences in the prevalence of resistance.

Table 3. Comparison of antimicrobial resistance among *Neisseria gonorrhoeae* in New Zealand and Australia

Antimicrobial	Percent (number) resistant:	
	NZ 2014-2015[1] *n = 425*	**AGSP 2014[2]** *n = 4804*
penicillin	12.0 (51)	29.0 (1370)
azithromycin	1.7 (7)	2.5 (119)
ciprofloxacin	32.2 (137)	36.0 (1750)
ceftriaxone decreased susceptibility (MIC 0.06–0.12 mg/L)	2.6 (11)	5.4 (258)
high-level tetracycline	15.8 (67)	19 (number not reported)
spectinomycin	0.0 (0)	0.0 (0)

1 Data from this survey.
2 Data from the Australian Gonococcal Surveillance Programme (AGSP, see reference 8).

Reassuringly, the prevalence of decreased susceptibility to ceftriaxone was low (2.6%), and none of the isolates with decreased susceptibility belonged to the globally disseminated ST1407 clone that has previously been associated with decreased ceftriaxone susceptibility.[9]

An important observation was that 11.1% of isolates were non-susceptible to azithromycin. In New Zealand, dual therapy with ceftriaxone and azithromycin is recommended for empiric treatment, so this finding is of concern, and warrants ongoing monitoring. Moreover, there was evidence of clonal spread of azithromycin non-susceptible isolates, with one clone, ST10193, being prevalent.

Similar to other studies, a diverse range of NG-MAST types were identified. For example, a 2009-2010 survey from the European Gonococcal Antimicrobial Resistance Surveillance Programme identified a total of 425 NG-MAST types among 1066 isolates, of which only 125 types occurred in ≥2 isolates. Interestingly, the three most prevalent types in Europe in 2009-2010 (ST1407, ST2992 and ST225) did not feature in the 30 most commonly detected NG-MAST types in New Zealand.[10]

This survey had some limitations. First, due to the limited amount of culturing for *N. gonorrhoeae* now undertaken in many New Zealand diagnostic laboratories as a result of the trend to nucleic acid-based diagnosis of gonorrhoea, gonococci circulating in regions outside the greater Auckland area were under-represented in this 'national' survey. This finding highlights the need for laboratories to maintain a level of gonococcal culture sufficient to monitor local resistance patterns. Second, because any enhanced culturing undertaken specifically for the duration of this survey focussed on obtaining specimens from patients attending sexual health clinics, the isolates we obtained for the survey may not have been an accurate representation of gonococcal strains circulating in a particular area.

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APPENDIX

Table 4. Number of isolates referred by each laboratory

Laboratory	Number of isolates	Percentage of total
Whangarei Hospital*	9	2.1
LabPlus*	130	30.6
Middlemore Hospital	1	0.2
Labtests*	164	38.6
Waikato Hospital*	27	6.4
Pathlab Waikato*	2	0.5
Laboratory Services, Rotorua*	2	0.5
Pathlab Bay of Plenty*	14	3.3
Tlab, Gisborne*	4	0.9
Southern Community Labs, Hastings*	15	3.5
Labcare Pathology, New Plymouth*	3	0.7
Medlab Central*	6	1.4
Wellington Hospital	2	0.5
Aotea Pathology*	8	1.9
Canterbury Health Laboratories*	26	6.1
Southern Community Labs, Dunedin*	11	2.6
Southern Community Labs, Invercargill*	1	0.2
Total	**425**	

* Denotes laboratories that were requested to refer gonococcal isolates for the survey. No isolates were received from four laboratories that were requested to refer isolates.

Table 5. Site of isolation of *Neisseria gonorrhoeae*

Site	Number (column percent)[1]:			
	Female		**Male**	
Urethral	0	(-)	227	(66.2)
Cervix/vagina	73	(91.3)	-	(-)
Anorectal	0	(-)	55	(16.0)
Penile	-	(-)	44	(12.8)
Urogenital (not otherwise specified)	6	(7.5)	4	(1.2)
Throat/pharynx	0	(-)	9	(2.6)
Urine	1	(1.3)	2	(0.6)
Other	0	(-)	2[2]	(0.6)
Total[1]	**81**		**344**	

1 Site of isolation not reported for two patients: 1 female and 1 male. These two patients are included in the totals for each gender, but not in the denominator used to calculate the percentages for each site.
2 Includes 1 isolate from the eye and 1 from a knee aspirate.

Table 6. Distribution of isolates referred for the survey by district health board and region

District health board / region	Number of isolates	Percentage of total
Northern	**304**	**71.5**
Northland	12	2.8
Waitemata/Auckland/Counties Manukau	292	68.7
Midland	**52**	**12.2**
Waikato	29	6.8
Lakes	2	0.5
Bay of Plenty	14	3.3
Tairawhiti	4	0.9
Taranaki	3	0.7
Central	**31**	**7.3**
Hawke's Bay	15	3.5
Whanganui	1	0.2
MidCentral	5	1.2
Capital & Coast/Hutt Valley	10	2.4
Wairarapa	0	-
Nelson Marlborough	0	-
Southern	**38**	**8.9**
West Coast	0	-
Canterbury/South Canterbury	26	6.1
Southern	12	2.8
Total	**425**	

Table 7. Distribution of minimum inhibitory concentrations (MICs) among *Neisseria gonorrhoeae* isolates

Antimicrobial	Percent of isolates with an MIC (mg/L) of:																
	0.002	**0.004**	**0.008**	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
penicillin – all					0.9	0.9	6.6	18.4	50.8	10.4	5.7	1.7	0.5	4.2			
β-lactamase +ve												4.8	9.5	85.7			
β-lactamase -ve					1.0	1.0	6.9	19.3	53.5	10.9	5.9	1.5					
cefixime	1.4	7.8	51.1	19.5	15.1	4.2	0.9										
ceftriaxone	7.3	46.1	23.3	8.7	12.0	2.4	0.2										
ertapenem	0.5	0.9	27.3	48.5	20.2	2.4	0.2										
ciprofloxacin	28.0	36.7	2.4	0.5	0.0	0.2	0.0	0.0	0.0	0.9	5.2	15.8	10.4				
tetracycline					0.5	1.7	17.4	12.5	24.5	17.4	8.7	0.2	1.4	9.7	6.1		
spectinomycin												0.2	1.9	32.2	64.2	1.4	
azithromycin					12.7	12.9	24.2	39.1	9.4	1.2	0.0	0.2	0.2				
gentamicin										0.2	1.9	31.3	64.9	1.7			

The white fields represent the range of antibiotic concentrations tested. MIC values less than or equal to the lowest concentration tested are presented as this lowest concentration. MIC values greater than the highest concentration tested are presented as the next highest concentration after the highest concentration tested. The vertical lines indicate the breakpoints between the susceptibility categories. For antibiotics where there are two vertical lines (ie, penicillin, ciprofloxacin, tetracycline, spectinomycin and azithromycin), the first line represents the breakpoint between susceptible and intermediate, and the second line represents the breakpoint between intermediate and resistant. For cefixime and ceftriaxone there is one vertical line which represents the breakpoint between susceptible and decreased susceptibility. For ertapenem and gentamicin there are no vertical lines as there are no interpretive standards for these antibiotics.

Table 8. NG-MAST types identified among the 399 *Neisseria gonorrhoeae* isolates typed

NG-MAST (ST)[1]	Number of isolates	Percentage of total
4186	45	11.3
2400	43	10.8
9368	27	6.8
10193	15	3.8
90018	14	3.5
90006	13	3.3
90019	12	3.0
7753	10	2.5
90010	10	2.5
7803	9	2.3
7175	8	2.0
10998	7	1.8
299	6	1.5
90020	6	1.5
356	5	1.3
4244	5	1.3
9166	5	1.3
90011	5	1.3
90023	5	1.3
90021	4	1.0
90022	4	1.0
90024	4	1.0
90042	4	1.0
5624	3	0.8
6950	3	0.8
7577	3	0.8
9650	3	0.8
11520	3	0.8
90033	3	0.8
90046	3	0.8
90052	3	0.8
217	2	0.5
402	2	0.5
1407	2	0.5

continued

NG-MAST (ST)[1]	Number of isolates	Percentage of total
2501	2	0.5
7268	2	0.5
8522	2	0.5
8570	2	0.5
8709	2	0.5
8883	2	0.5
9909	2	0.5
11760	2	0.5
90003	2	0.5
90005	2	0.5
90009	2	0.5
90012	2	0.5
90013	2	0.5
90026	2	0.5
90037	2	0.5
90045	2	0.5
90048	2	0.5
90050	2	0.5
90055	2	0.5
90065	2	0.5
28	1	0.3
697	1	0.3
766	1	0.3
1037	1	0.3
2992	1	0.3
3356	1	0.3
4995	1	0.3
5595	1	0.3
6339	1	0.3
6360	1	0.3
8028	1	0.3
8523	1	0.3
8598	1	0.3
8901	1	0.3
9471	1	0.3
10192	1	0.3
11095	1	0.3

continued

NG-MAST (ST)[1]	Number of isolates	Percentage of total
11371	1	0.3
11405	1	0.3
11422	1	0.3
11517	1	0.3
11746	1	0.3
11819	1	0.3
90001	1	0.3
90002	1	0.3
90004	1	0.3
90007	1	0.3
90008	1	0.3
90014	1	0.3
90015	1	0.3
90016	1	0.3
90017	1	0.3
90025	1	0.3
90027	1	0.3
90028	1	0.3
90029	1	0.3
90030	1	0.3
90031	1	0.3
90032	1	0.3
90034	1	0.3
90035	1	0.3
90036	1	0.3
90038	1	0.3
90039	1	0.3
90040	1	0.3
90041	1	0.3
90043	1	0.3
90044	1	0.3
90047	1	0.3
90049	1	0.3
90051	1	0.3
90053	1	0.3
90054	1	0.3
90056	1	0.3

continued

NG-MAST (ST)[1]	Number of isolates	Percentage of total
90057	1	0.3
90058	1	0.3
90059	1	0.3
90060	1	0.3
90061	1	0.3
90062	1	0.3
90063	1	0.3
90064	1	0.3
90066	1	0.3

1 NG-MAST types commencing with '900' (ie, number format '900nnn') are temporary types that have been assigned as the *por* and *tbpB* alleles identified, or the combination of *por* and *tbpB* alleles, were not found in the NG-MAST database.

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