Molecular detection of Neisseria gonorrhoeae resistance in a Berlin MSM cohort

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AIM

An increase in antibiotic resistance in Neisseria gonorrhoeae (NG) can be observed worldwide. One group of persons with an above-average infection rate is the MSM community. We analyzed random selected samples of positive NG swabs from MSM to test for resistance.

15 cultured NG patient samples with microbiologically tested antibiogram (etest), 2 ATCC-strains and 77 fresh swabs tested positive for NG using the Seegene AllplexTM STI Essential Assay (detecting *Chlamydia trachomatis* (CT), Mycoplasma hominis (MH), Trichomonas vaginalis (TV), Ureaplasma urealyticum (UU), Mycoplasma genitalium (MG), Neisseria gonorrhoeae (NG) and *Ureaplasma parvum* (UP). Of the fresh swabs, 40 showed at least one, in some cases up to three, other STI (including the AllplexTM Genital ulcer Assay, detecting Cytomegalovirus (CMV), Herpes simplex virus type 1 and 2 (HSV1) HSV2), Lymphogranuloma venereum (LGV), Haemophilus ducreyi (HD) and Treponema pallidum (TP). All samples were tested for azithromycin resistance (A2059G, C2611T) and ciprofloxacin resistance (S91F) using the AllplexTM NG & DR assay. Furthermore, the positions in the gyrA (91-95) and parC (86-88) gene (ciprofloxacin-resistance) were analyzed using an LDT multiplex PCR; the probes used in this case detect wild type. The multiplex LDT additionally detects the presence of penicillin-binding-protein (PBP-2) and penicillinase-producing NG (PPNG). For another comparison we sequenced a part of the sample using on the NG-STAR method published standardized (https://ngstar.canada.ca/). It offers the classifying of seven well-characterized genes associated antimicrobial resistance in N. gonorrhoeae (penA, mtrR, porB, ponA, gyrA, parC and 23S rRNA) to three classes of antibiotics (cephalosporins, macrolides and fluoroquinolones).

RESULTS

Two of 94 samples could not be confirmed in the Allplex™ NG & DR assay neither in the LDT, probably due to low bacteria amount. 66 of 90 patient samples showed an S91F mutation in the Allplex-assay, all of which were confirmed in the LDT. All isolates culturally determined as ciprofloxacin resistant were confirmed in the PCRs. One sample sensitive in culture showed a ciprofloxacin resistance in the Allplex- and LDT assay. Unfortunately, we have no sequencing data for the gyrA gene for this sample. The LDT detected additional mutations in gyrA in 10 fresh samples, which were probably not the S91F mutation. Since a negative PCR result is decisive for the detection of gyrA resistance and there is no NG positive control in the LDT, these results should be carefully checked if the amount of bacteria is low. In sequencing the leading gyrA mutation was the S91F, often accompanied by D95A. Only one fresh sample showed azithromycin resistance in the Allplex but no mutations in sequencing. Of two azithromycin resistant samples detected in culture, one was confirmed by the Allplex-PCR and sequencing, the other neither nor. One sample tested sensitive in culture was found to be resistant to ciprofloxacin in both PCRs. The LDT additionally showed 16 fresh samples positive for PPNG and 6 positive for PBP-2 mutations.

CONCLUSIONS

Our random sample of 77 fresh NG positive swabs showed a very high proportion of ciprofloxacin resistance (70%) with high agreement between the two PCR methods and the sequencing. Compared to resistance data in Germany this fits good, but only one fresh sample was detected with azithromycin resistance, an underdetection analyzing only two point mutations with the Allplex assay. In 3 out of 9 sequenced fresh samples the mutation A39T in MTR was detected, that also correlates to Azithromycin resistance. Overall, only 9 (12%) of all positive NG confirmed samples showed no resistance at all.

Acknowledgements

The consumables for AllplexTM STI Essential and AllplexTM NG & DR assays were funded by Seegene.

Seegene pret	test	in house m	ultinlex PCI	R (ct-va	lues)	Seegen	e Allnley	™ NG &	DR as	sav	culture	Sequencing
	NG	Cephalosporine	 			Ciprofloxacin		omycin		NG2	resistance	NG-Star project
	ct-value	PBP-2	PPNG	parC	gyrA	S91F	A2059G	C2611T	NG1		e-test	V4 (T
ATCC 49226 ATCC 43069	11,31 8,68	-	- 15,07	17,24 15,18	16,58 14,27	_	-	-	16,61 13,09	14,43 12,04	sensitive not tested	WT WT
NG cultured	11,32	-	-	-	-	19,2	-	-	16,43	14,64	cipro	S91F, D95A gyrA
NG cultured	11,67	-	17,63	22,4	-	17,53	-	-	14,83	13,51	cipro	S91F, D95A gyrA
NG cultured NG cultured	8,2 11,2	16,41	11,54 -	-	-	16,53 18,01	- -	-	13,82 15,28	11,83 13,3	cipro cipro	S91F, D95A gyrA, L421P ponA S91F, D95A
NG cultured	10,72	-	7,54	15,97	15,36	-	-	-	13,71	11,82	Azi	WT
NG cultured	11,1	-	8,51	-	-	16,89	-	-	14,32	13,24	cipro	S91F, D95A gyrA, L421P ponA
NG cultured	8,59	-	15,28	-	-	15,61	-	-	12,98	11,23	cipro	A39T mtr
NG cultured NG cultured	8,99 11,33	-	- -	20,23	-	15,53 16,67	- -	-	13,04 13,98	11,51 11,79	cipro sensitive	A39T mtr
NG cultured	8,45	-	-	-	-	13,99	-	-	11,56	10,59	cipro	A39T mtr
NG cultured	8,18	-	-	-	-	15,75	-	-	13,07	12,1	cipro	A39T mtr.
NG cultured	8,57	-	-	-	-	15,98	-	-	13,36	11,87	cipro	A39T mtr
NG cultured NG cultured	8,5 9,64	16,66	- -	20,55	- 19,77	15,95	-	-	13,48 14,41	11,94 12,49	cipro sensitive	mtr WT mtr WT
NG cultured	9,69	-	- -	20,33	19,77	-	14,18	_	13,55	11,59	Azi	A2059G Azi
NG, MH, CT	24,46	-	28,02	27,37	-	32,6	-	-	30,55	28,01		
UU, NG, MH	27,91	-	33,95	29,03	-	35,81	-	-	33,23	31,12		14040
UU, NG NG	15,21	-	19 18,52	21,73 27,23	21,5 27.41	-	-	-	20,43 27,87	17,93 26,09		L421P ponA
NG, MH	21,26 21,26	-	16,76	-	27,41 -	29,02	- -	- -	26,58	24,62		
NG	16,67	_	18,19	25,61	_	24,32	_	_	22,29	19,77		S91F, D95A gyrA + A39T mtr
UU, NG	34,12	_	35,11	38,48	_	42,85	_	_	39,74	37,52		391F, D93A gyrA + A391 IIIII
NG, CT	26,11	-	17,99	28,76	-	34,17	-	-	31,75	29,15		
NG	22,34	-	-	35,19	-	30,19	-	-	27,71	25,57		
UU, NG, MH	23,19	38,29	31,94	23,04	-	31,02	-	-	28,82	26,33		
UU, NG, CT UU, NG	25,5 25,77		-	30,32 30,56	-	33,13 32,95	- -	-	30,55 30,86	28,44 29		
NG, CT	25,77 18,98	-	24,29	JU,JU -	-	26,52		-	24,47	29 21,88		L421P ponA; S91F, D95A gyrA; A39T mtr
UU, NG	17,17	-	-	-	-	23,66	-	-	21,28	19,55		S91F, D95A gyrA
NG	20,5	-	-	-	-	28,73	-	-	26,18	23,85		
UU, NG	19,45	25,89	-	-	-	27,55	-	-	25,12	21,56		
NG NG, MG	20,67 19,92		-	28,17 31,38	30,18	28,44	- -	-	25,85 25,8	24,15 23,84		
NG NG	20,24	_	-	-	_	27,87	_	-	25,51	23,5		
UU, NG	37,64	-	-	-	-	-	-	-	-	-		
NG	19,2	-	-	27,02	28,99	-	-	-	24,24	22,68		
UU, NG, MG	28,27	-	-	31,57	-	24.07	-	-	33,76	31,99		SO1E DOEA ourA
UU, NG, MH NG	17,11 25,29	-	31,31	32,5	-	24,87 32,79	_	- -	22,44 31,19	20,61 28,83		S91F, D95A gyrA
UU, NG, MG	19,11	-	-	26,07	_	-	25,63	-	24,36	22,03		S91l gyrA
NG	24,79	-	-	-	-	31,14	-	-	28,89	27,02		
NG	29,55	-	-	32,12	-	37,96	-	-	35,79	33,19		
NG NG	33 21 17	-	-	-	_	- 20.00	- <u>-</u>	-	- 26.62	36,95 24,43		
NG	21,17 21,61	_	- -	28,1	-	29,09 30,43	- -	-	26,62 27,92	25,94		
NG	17,69	33,98	-	23,24	-	24,89	-	-	22,56	20,66		S91F, D95A gyrA
NG	22,96	-	-	33,21	-	30,74	-	-	28,29	26,02		
NG	32,66	-	- 25.20	37,27	-	-	-	-	36,94	33,8		6045 DOFA A A20T .
NG NG	18,86 16,52	-	25,39 22,4	26,82 24,06	-	26,3 24,32	-	-	24,04 21,82	22,39 19,76		S91F, D95A gyrA; A39T mtr S91F, D95A gyrA
NG, MG, CT	22,65	-	- -	30,2	35,43	- 24,32	- -	- -	28,36	25,84		SEIF, DESA GYIA
NG	21,37	-	-	-	-	29,28	-	-	26,98	24,66		
NG	19,04	-	-	31,06	-	28,41	-	-	25,94	23,62		
NG	23,68	-	-	31,34	44,72	-	-	-	28,97	27,44		
UU ,NG NG	29,59 17,54	-	-	_	-	35,29 25,61	-	-	35,35 23,16	32,42 21,56		
NG	34,67	-	-	38,36	-	-	-	-	37,88	37,36		
NG	18,16	-	-	26,62	30,83	-	-	-	23,74	21,58		
NG	19,6	-	-	27,24	31,37	-	-	-	25,44	23,73		
NG, MH, MG	24,27	-	-	33,24	-	32,32	-	-	30,05	28,2		
NG, MH NG, CT	27,71 17,13	23,51	- <u>-</u>	31	-	35,75 25,63		-	33,86 23,3	31,65 21,11		
NG, C1	22,98	-	_	-	-	30,76	_	-	28,25	25,99		
NG, MH	20,47	-	-	-	-	27,7	-	-	25,12	23,4		
UU, NG	22,1	-	-	-	-	29,9	-	-	27,76	25,54		
NG NG MH	25,19 20 /	-	28,04	31,87 27.66	-	33,13	-	-	30,38	27,81 23.5		
NG, MH UU, NG, MH, CT	20,4 20,18	-	-	27,66 -	-	28,41	- -	-	25,68 25,7	23,5 23,27		
NG	24,57	-	-	-	-	32,21	-	-	29,31	27,81		
NG	16,85	-	-	24,93	-	28,96	-	-	26,17	23,78		
UU, NG, CT	23,41	-	-	29,48	37,02	-	-	-	28,04	25,69		
NG	14,89 21.00	-	-	- 32 00	-	22,29	-	-	19,41 25,62	17,26 22.69		
UU, NG, CT NG	21,09 23,65	-	33,56	32,99 24,95	-	28,26 33,55	- -	-	25,62 28,77	22,69 26,73		
UU,NG, MG	24,57	_	-	33,13	-	32,65	-	-	30,14	27,84		
NG	24,64	30,88		37,9	-	32,46	-	-	29,92	27,81		
NG NG GT	34,64	-	-	-	- 22.15	-	-	-	-	-		
NG, CT NG, MG	24,96 28,88		-	34,82 33,89	33,15	36,43	- -	-	29,58 34,22	26,15 31,43		
UU, NG, MH, MG	34,32	-	-	36,07	-	-	-	-	37,34	36,33		
NG, UP	25,39	-	-	35,5	-	32,91	-	-	30,56	28,95		
NG	30,68	-	-	34,5	-	-	-	-	36,4	35,32		
UU, NG NG, HSV2	19,29 18,11	-	- -	-	-	27,39 27,32	-	-	24,61 24.54	22,15 22,1		
NG, HSV2 NG	18,11 17,37	_	-	-	-	27,32		-	24,54 22,01	22,1 19,88		
NG, MH, CT, HSV1	15,46	_	-	-	-	23,06	-	-	20,28	18,07		
NG, MH, MG, HSV2	25,76	-	31,36	34,49	32,65	-	-	-	30,56	27,85		
NG,CT, CMV	22,12	21.00	-	41,02	-	29,77	-	-	27,22	24,41		
UU, NG, MH, MG NG, CT	31,53 29,62	31,66	- -	34,09 37,55	-	38,22	- -	-	37,33 35,5	33,44 33,71		
NG NG	14,73	-	-	-	-	22,01	-	-	19,29	17,26		
NG	33,67	-	-	36,68	-	-	-	-	37,94	35,51		
7E confirmed to	NC ·											Cognoncing recults not for act.
75 confirmed swabs % resistance	+ טעו	8	21,3	32	81,3	72	1,3					Sequencing results not for mtr Sequencing results only for mtr
73 T COISCUITCC			21,0	32	31,3	, _	1,5				I	Complete sequencing results

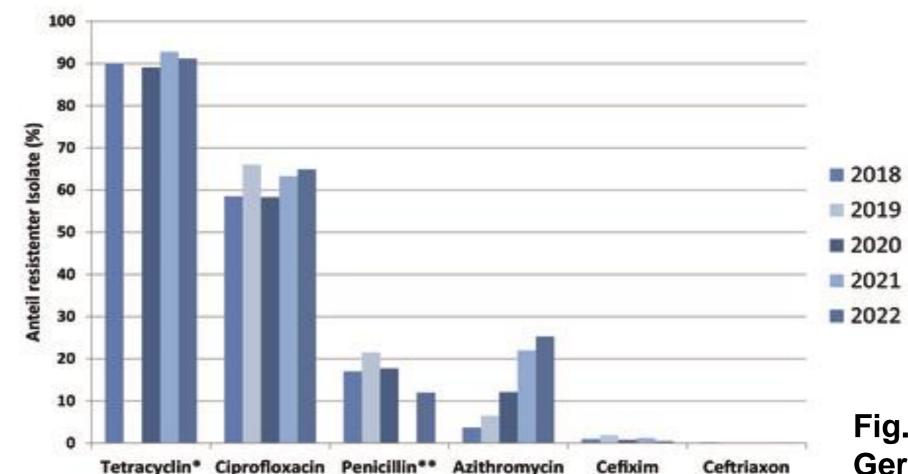


Fig. 1: N. gonorrhoeae resistance data in Germany published by the Robert Koch Institute

