

Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix to Manuscript Entitled
Doxycycline to prevent bacterial sexually transmitted infections

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Supplemental Analyses:

Per protocol analyses

Two per protocol analyses were conducted.

- 1) Per protocol analysis excluding those with doxycycline discontinuation.

In a prespecified analysis, for each cohort the doxycycline PEP arm was restricted to study time prior to the first discontinuation of study drug by self-report or documented on the study CRFs. A discontinuation was defined as a clinician-directed product hold or a participant-reported cessation of doxycycline usage lasting for more than a week. Doxycycline PEP was temporarily held during STI treatment with doxycycline (e.g., for *Chlamydia trachomatis* diagnosis) and was not considered as a discontinuation. In an analysis limited to participants who did not have doxycycline discontinued (1233 quarters, 467 participants: 320 doxy-PEP, 148 SOC), the relative reduction of incident STI's per quarter with doxy-PEP was 0.32 (95%CI 0.23, 0.44) per quarter in the PrEP cohort and 0.37 (95%CI:0.23, 0.59) in the PLWH cohort.

- 2) Per protocol analyses by self- reported adherence

In an analysis limited to participants who reported always taking doxycycline after sex (1002 quarters, 413 participants: 265 doxy-PEP, 148 SOC) the relative reduction of incident STI's per quarter with doxy-PEP was 0.26 (95%CI 0.18, 0.38) per quarter in the PrEP cohort and 0.32 (95%CI: 0.19, 0.54) in the PLWH cohort.

Recurrent and multiple STIs

Among SOC participants, 37.3% (77/206) had at least one quarter with ≥ 1 STIs diagnosed, and 21.4% (44/206) were diagnosed with STIs in more than one quarter. Of SOC participants who had a primary STI endpoint, 20% (24/121) had multiple STIs diagnosed during the quarter with a primary endpoint. In the doxy-PEP arm, 18.8% (81/431) had at least one quarter with ≥ 1 STIs diagnosed, and 3.7% (16/431) had STIs diagnosed in more than one quarter. Among those assigned to doxy-PEP who had a primary STI endpoint, 6.2% (6/97) had more than one STI diagnosed during the quarter with a primary endpoint.

Figure 1. Primary endpoint of one or more STI per quarter by individual STI
Note: Randomization was 2:1 doxy-PEP vs. SOC. There was a significant reduction in STI incidence with doxyPEP despite a similar number of endpoints in the doxy-PEP and SOC arms

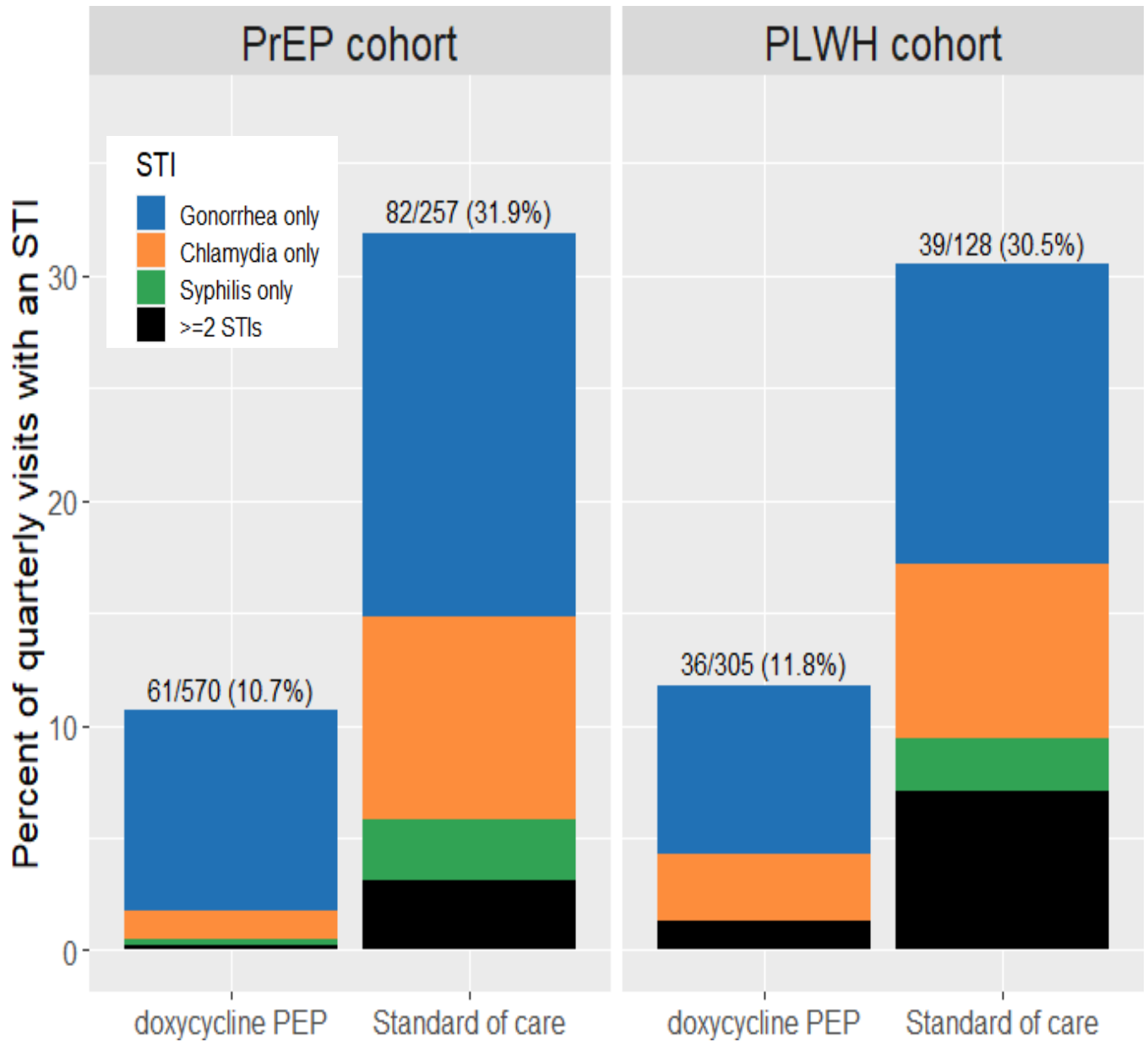


Figure 2. Anatomic distribution of incident STIs.

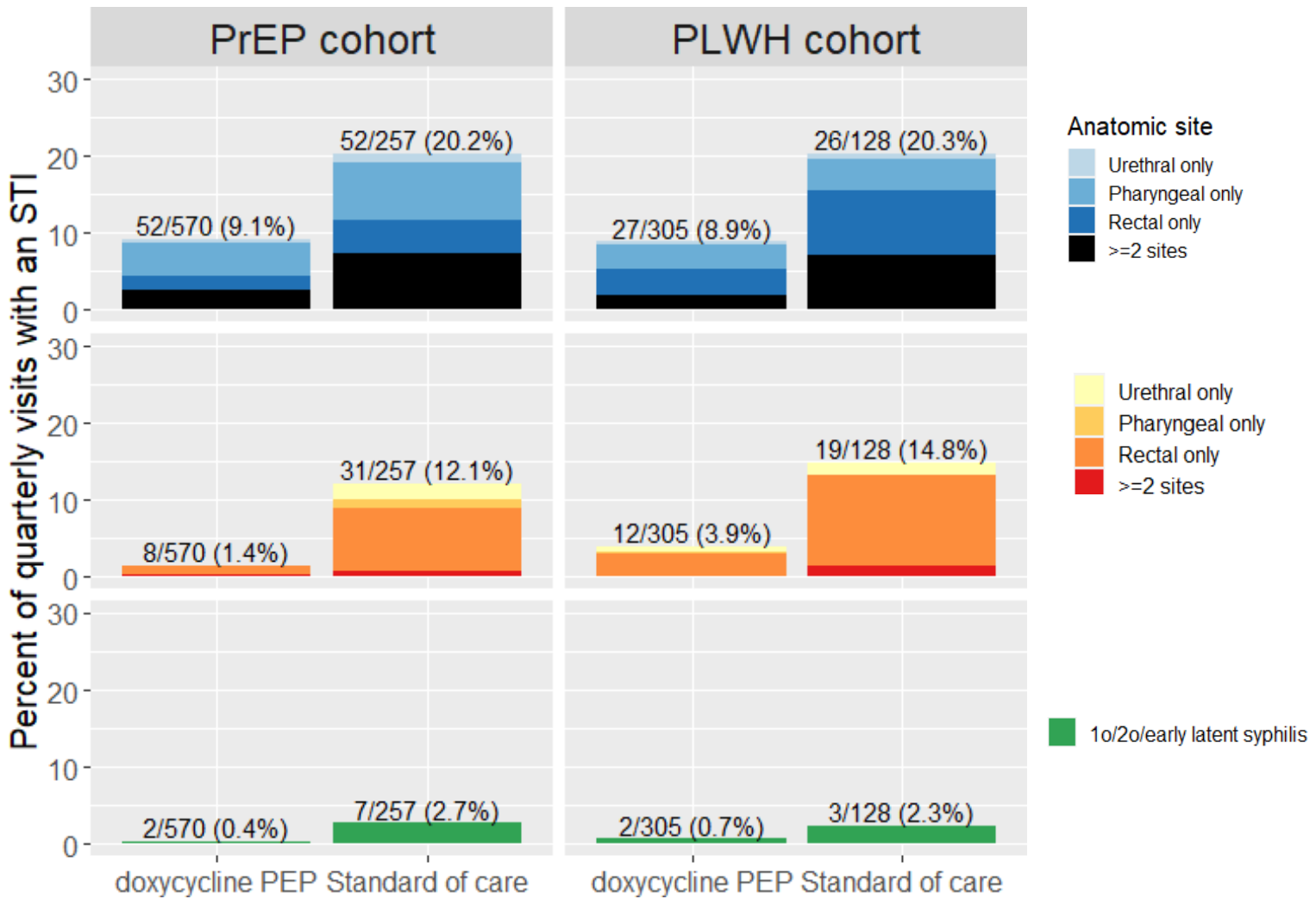


Table 1. Proportion of culture-positivity and tetracycline antimicrobial resistance in participants diagnosed with *N. gonorrhoeae* infection

		PrEP		PLWH		Total	
		Doxy PEP	SOC	Doxy PEP	SOC	Doxy PEP	SOC
Baseline	GC infections	40	20	25	14	65	34
	GC cultures with resistance testing available	5	4	2	4	7	8
	Tetracycline-resistant	2 (40.0%)	1 (25.0%)	0 (0%)	1 (25.0%)	2 (28.6%)	2 (25.0%)
On Study	GC infections	52	52	27	26	79	78
	GC cultures with resistance testing available	7	10	6	6	13	16
	Tetracycline-resistant	3 (42.9%)	1 (10.0%)	2 (33.3%)	1 (16.7%)	5 (38.5%)	2 (12.5%)

Table 2. Proportion of culture-positivity and doxycycline resistance in *S. aureus* isolates from baseline and follow-up by arm

		PrEP		PLWH		Total	
		Doxy PEP	SOC	Doxy PEP	SOC	Doxy PEP	SOC
Baseline	Total samples	209	104	117	53	326	157
	<i>S. aureus</i> growth	83	46	56	30	139	76
	Doxycycline-resistant	8 (9.6%)	7 (15.2%)	4 (7.1%)	6 (20%)	12 (8.6%)	13 (17.1%)
	MSSA ¹	73	43	45	24	118	67
	Doxycycline-resistant	7 (9.6%)	7 (16.3%)	4 (8.9%)	2 (8.3%)	11 (9.3%)	9 (13.4%)
	MRSA ²	9	3	11	6	20	9
	Doxycycline-resistant	1 (11.1%)	0 (0%)	0 (0%)	4 (66.7%)	1 (5.0%)	4 (44.4%)
Month 6	Total samples	122	47	70	28	192	75
	<i>S. aureus</i> growth	34	16	17	13	51	29
	Doxycycline-resistant	10 (29.4%)	1 (6.3%)	1 (5.9%)	2 (15.4%)	11 (21.6%)	3 (10.3%)
	MSSA	30	15	12	11	42	26
	Doxycycline-resistant	9 (30.0%)	1 (6.7%)	1 (8.3%)	1 (9.1%)	10 (23.8%)	2 (7.7%)
	MRSA	4	1	5	2	9	3
	Doxycycline-resistant	1 (25.0%)	0 (0%)	0 (0%)	1 (50.0%)	1 (11.1%)	1 (33.3%)
Month 12	Total samples	74	37	37	14	111	51
	<i>S. aureus</i> growth	21	17	10	7	31	24
	Doxycycline-resistant	4 (19.0%)	1 (5.9%)	1 (10.0%)	1 (14.3%)	5 (16.1%)	2 (8.3%)
	MSSA	20	15	9	6	29	21
	Doxycycline-resistant	4 (20.0%)	1 (6.7%)	1 (11.1%)	0 (0%)	5 (17.2%)	1 (4.8%)
	MRSA	1	2	1	1	2	3
	Doxycycline-resistant	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (33.3%)

¹ MSSA: Methicillin sensitive *S. aureus*

² MRSA: Methicillin resistant *S. aureus*

Table 3. Laboratory monitoring of CBC and liver function tests

	Doxy-PEP ¹		Standard of care
	Month 3	Month 9	First observation ²
Laboratory results (median [IQR])	308	198	74
Complete blood count			
Total leukocyte count (x10 ⁹ /L)	5.8 (4.94–6.97)	6.01 (5.09–6.9)	5.94 (5.03–7)
Absolute neutrophil count (x10 ⁹ /L)	3.14 (2.52–3.87)	3.14 (2.52–3.91)	3.08 (2.38–4.01)
Total hemoglobin (g/dL)	15.1 (14.4–15.7)	15 (14.4–15.6)	15.3 (14.4–16)
Platelet count (x10 ⁹ /L)	253.5 (220–295)	255 (217–286)	250 (222–297)
Liver function tests			
Total bilirubin (mg/dL)	0.6 (0.5–0.8)	0.6 (0.4–0.7)	0.6 (0.6–1.5)
AST (SGOT) (IU/L)	24 (20–29)	25 (21–30)	23 (19–24)
ALT (SGPT) (IU/L)	24 (19–31)	25 (19–32)	24 (18–33)
Alkaline phosphatase (IU/L)	69 (57–81)	70 (58.5–84)	63 (56–78)

- 1 CBCs and LFTs were scheduled to be done at month 3 and month 9 visit for participants in the doxy-PEP arm; if a participant missed that visit, CBCs and LFTs were scheduled to be made up at month 6 and month 12 visits, respectively. Make-up samples are included in month 3 and 9 scheduled collection here.
- 2 CBCs were scheduled to be done at least once during follow-up. If testing was done more than once, only the first sample was used in this analysis. LFTs were not conducted as part of DoxyPEP for participants in the standard of care arm; if LFTs were reported as part of standard of care they are included here (N = 7).

Table 4. Adverse Events

Reporting criteria	Event	Determination of relationship
Grade ≥ 2 lab abnormality related to doxycycline	Elevated ALT (<i>Grade 2</i>)	Possible
Grade ≥ 3 adverse event related to doxycycline	Diarrhea (<i>Grade 3</i>)	Probable
	Diarrhea (<i>Grade 3</i>)	Possible
	Diarrhea (<i>Grade 3</i>)	Possible
	Headache/migraine (<i>Grade 3</i>)	Possible
	Headache/migraine (<i>Grade 3</i>)	Possible
Serious adverse event	Cat bite (req. hospitalization)	Unrelated
	Testicular torsion (req. surgery)	Unrelated
	Hypotension 2o to substance use (req. hospitalization)	Unrelated
	Gastroenteritis (req. hospitalization)	N/A (not in doxy-PEP arm)

Table 5. Symptoms reported at quarterly visits

	Doxy-PEP		Standard of Care
	Proportion of quarters with symptoms reported	Proportion of quarters with symptoms reported & attributed to doxy-PEP by clinician	Proportion of quarters with symptoms reported
Skin rash	57/928 (6.1%)	5/928 (0.5%)	23/402 (5.7%)
More readily sunburned	39/928 (4.2%)	21/928 (2.3%)	4/402 (1.0%)
Headache	76/928 (8.2%)	1/928 (0.1%)	37/402 (9.2%)
Changes in vision	22/928 (2.4%)	1/928 (0.1%)	10/402 (2.5%)
Pain with swallowing	20/928 (2.2%)	0/928 (0.0%)	13/402 (3.2%)
Difficulty swallowing	13/928 (1.4%)	1/928 (0.1%)	11/402 (2.7%)
Diarrhea	112/928 (12.1%)	26/928 (2.8%)	37/402 (9.2%)
Nausea	62/928 (6.7%)	14/928 (1.5%)	13/402 (3.2%)
Vomiting	23/928 (2.5%)	3/928 (0.3%)	12/402 (3.0%)
Abdominal pain	38/928 (4.1%)	2/928 (0.2%)	15/402 (3.7%)
Other	57/928 (6.1%)	1/928 (0.1%)	30/402 (7.5%)

Table 6. Baseline characteristics of study participants living with HIV

Baseline characteristics of PLWH	N=174
N (%) with documented ART usage	173 (99.4%)
Median (IQR) CD4 ⁺ count (cells/mm ³)	697.5 (499–880)
N (%) with HIV viral load ≤ 50	158 (94.6%)
Median (IQR) viral load among viremic participants (N=9)	1354 (145–7123)

	N=174
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N (%) with HIV viral load ≤ 50	158 (94.6%)
Median (IQR) viral load among viremic participants (N=9)	1354 (145–7123)

Table 7. Representativeness of the participant sample

Category	Example
Disease, problem or condition under investigation	Bacterial sexually transmitted infections (STIs) in men who have sex with men (MSM)
Special Considerations related to:	
Sex & Gender	STIs disproportionately impact men who have sex with men[1], with a marked impact on those who are living with HIV[2, 3] or who are taking HIV PrEP[4, 5]. In the US, the plurality of new syphilis diagnosis are in MSM (46%). Chlamydia is more common in women than men, whereas gonorrhea is more common in men than women. However, in people accessing STI clinics, MSM were more commonly diagnosed with chlamydia and gonorrhea than women or men who have sex with women.
Race or ethnic group	Some racial and ethnic groups are more impacted by STIs. In 2021 CDC data[1], non-Hispanic Black/African Americans had the highest case rates of chlamydia and gonorrhea, compared to other racial and ethnic groups, and syphilis was most common in American Indian/Alaska Native and Black/African-Americans.
Geography	The STI epidemic is global[6], with a disproportionate impact described in MSM in the US[1], Australia[7] and Western Europe[8].
Other considerations	
Overall representativeness of this trial	This study enrolled 8% Black, 11% Asian/Pacific Islander, 15% multiple races/other, and 65% White. 30% of participants identified as Hispanic/Latinx. The proportion of black participants were slightly lower than current US population distribution[9], whereas Latinx enrollment exceeded current US Latinx population percentages. Overall, the study enrolled a diverse population that is representative of the US population. Transgender women/gender diverse participant were 4% of the study, an insufficient proportion to draw conclusions about doxy-PEP efficacy in this population. The study enrolled in Seattle and San Francisco, two urban US West Coast cities, thus do not have data from other geographic locations.

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