What's left in the cupboard? Older antimicrobials for treating gonorrhoea

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Synopsis

Background

Neisseria gonorrhoeae has developed resistance to all antimicrobials used to treat gonorrhoea, with even ceftriaxone being undermined. It is therefore important to examine any potential to redeploy older antimicrobials routinely used for other infections to treat ceftriaxone-resistant gonococcal infections.

Objectives

We examined the susceptibility of *N. gonorrhoeae* to aztreonam, chloramphenicol, co-trimoxazole, fosfomycin, piperacillin/tazobactam and rifampicin.

Materials and Methods

N. gonorrhoeae isolates (n=94) were selected to include a range of antimicrobial susceptibilities: 58 were collected in the Gonococcal Resistance to Antimicrobials Surveillance Programme; 17 were clinical isolates referred to the PHE reference laboratory, and 19 were control strains. MICs were determined by agar dilution for the six study antimicrobials, and for ceftriaxone and azithromycin as comparators.

Results

There was correlation between piperacillin/tazobactam and ceftriaxone MICs, but all five isolates with high ceftriaxone MICs (>0.5 mg/L) were inhibited by piperacillin/tazobactam at 0.06-0.5 mg/L. Aztreonam MICs for ceftriaxone-resistant isolates exceeded those of ceftriaxone. Among non-β-lactams, fosfomycin and co-trimoxazole had low, tightly-clustered MICs suggesting widespread susceptibility; rifampicin split the collection into highly-susceptible and highly-resistant groups; chloramphenicol had a wide MIC distribution.

Conclusions

Although unsuitable for empirical use, piperacillin/tazobactam, fosfomycin, co-trimoxazole, rifampicin and, possibly, chloramphenicol could be considered for individual patients with ceftriaxone-resistant gonococcal infection once MICs are known. Wider surveillance of the susceptibility of *N. gonorrhoeae* to these agents is needed, along with clinical trials and the establishment of clinical breakpoints for *N gonorrhoeae*.

Introduction

Neisseria gonorrhoeae, the causative pathogen of gonorrhoea, has developed resistance to successive classes of antibiotics.[1] Few antimicrobials remain widely effective for treatment, which now largely depends upon extended-spectrum cephalosporins (ESCs), principally ceftriaxone, alone or combined with azithromycin. Of great concern, therefore, is the international spread of the extensively-drug-resistant *N. gonorrhoeae* FC428 clone,[2] associated with ceftriaxone resistance and raised MICs for azithromycin. In addition, non-FC428 *N. gonorrhoeae* with ceftriaxone resistance and high-level azithromycin resistance were detected in both England and Australia in 2018.[3] Two cases in England failed treatment with ceftriaxone and eventually were cured with three days of intravenous ertapenem.[4, 5]

There is a dearth of treatment options for patients who cannot be treated with ESCs (or, potentially, ertapenem) owing to severe allergy. Established non-β-lactam therapies such as azithromycin, ciprofloxacin and tetracycline have unacceptably high rates of resistance for empirical use,[6] and if susceptibility is tested, isolates often prove resistant. Spectinomycin is widely active but is no longer available in many countries; gentamicin is useful for genital and anal infections,[7] but has a high failure rate in pharyngeal infections.

One strategy to increase the number of treatment options in cases of resistance or allergy is to redeploy older antimicrobials that are not routinely used to treat gonococcal infections. Here, we examined the possible utility of aztreonam, chloramphenicol, co-trimoxazole, fosfomycin, piperacillin/tazobactam and rifampicin.

Materials and Methods

N. gonorrhoeae isolates

A total of 94 *N. gonorrhoeae* isolates were selected: 58 were collected during 2012-2016 as part of the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP);[8] 17 were clinical isolates that had been referred to the PHE reference laboratory, generally owing to unusual resistance, and 19 were controls, including the 14 WHO reference strains.[9]

The panel was selected to include isolates with a range of resistances variously to penicillin (chromosomally-mediated and plasmid-mediated), cefixime, ceftriaxone, azithromycin (moderately and highly raised MICs), ciprofloxacin, tetracycline (chromosomally-mediated and plasmid-mediated) and spectinomycin; it also included isolates that were fully susceptible to all of these antimicrobials.

All archived isolates were retrieved from -80°C and inoculated on to non-selective GCVIT agar (GC agar base (Becton, Dickinson and Co, Le Pont de Claix, France) containing 1% Vitox (Oxoid, Basingstoke, UK)). Inoculated plates were incubated at 36°C in 5% CO_2 for 18-24 h. Growth was sub-cultured on to GCVIT agar plates and incubated again at 36°C in 5% CO_2 for 18-24 h. Identification of isolates as *N. gonorrhoeae* had previously been performed by real-time PCR using *opa* and *porA* targets,[10] or by MALDI-ToF.

Antimicrobial susceptibility testing

Isolates were tested by the GRASP agar dilution method[8] using Diagnostic Sensitivity Test agar (HiMedia Laboratories GmbH, Einhausen, Germany) to determine MICs of: aztreonam (range 0.016-16 mg/L), chloramphenicol (0.016-32 mg/L), co-trimoxazole (1:19 ratio of trimethoprim 0.016-16 mg/L and sulfamethoxazole 0.3-304 mg/L), fosfomycin (2-64 mg/L), piperacillin/tazobactam (piperacillin at 0.15-4 mg/L and tazobactam at 4 mg/L), and rifampicin (0.06-16 mg/L). Azithromycin (0.06-4 mg/L) and ceftriaxone (0.004-0.25 mg/L) were included as comparators.

MICs were read after 48h incubation at 36°C in 5% CO_2.[8] Any isolates for which MICs for azithromycin and ceftriaxone exceeded the initial dilution range were retested by Etest (bioMérieux, Basingstoke, UK) (Table 1). GCVIT agar was used for the Etests and the agar plates were incubated at 36°C in 5% CO_2 for 24 h. The presence of β-lactamase was established by the Nitrocefin test (Oxoid).

Data analysis

Pearson's correlation coefficient (*R*) was used to test relationships between the log MICs of different antibiotics, and the associated P-value was calculated to test for significance; $P<0.05$ was used to indicate evidence of a relationship. For this analysis, 'off-scale' MIC values were taken as the next in-series dilution (i.e. >8 mg/L was assumed to be 16 mg/L and ≤0.5 was assumed to be 0.5 mg/L). Relating MICs of alternative agents to those of azithromycin presents two challenges: (i) that some isolates have extremely high levels of azithromycin resistance, with MICs >256 mg/L and (ii) that resistance is mechanistically diverse, with high-level resistance entailing 23S rRNA mutations unlikely to affect non-macrolides and low-level resistance substantially involving efflux changes that may have a wider effect.[1] Accordingly, two analyses were performed. In the first, azithromycin MICs ≥ 256 mg/L were edited to 16 mg/L to avoid outlier MICs that may skew correlations. In the second analysis, to investigate specifically the effect of cross-resistance due to upregulated efflux, we excluded isolates with azithromycin MICs >2 mg/L (where 23S rRNA mutations are likely)[1] and re-calculated *R*. Ceftriaxone resistance was regarded as an MIC >0.125 mg/L; EUCAST no longer has breakpoints for azithromycin and we took account of both the previous value of >0.5 mg/L and the ECOFF of 1 mg/L.[11]

Results

The isolate panel was chosen to include multi-resistant *N. gonorrhoeae*: 8/93 isolates were resistant to ceftriaxone at 0.125 mg/L and 20/93 to azithromycin at 0.5 mg/L (16/93 at the ECOFF of 1 mg/L).

MIC distributions of the test agents are shown in Table 1, whilst their activity against the ceftriaxone -resistant isolates, and those with azithromycin MICs >0.5 mg/L, is line-listed in Table 2.

For piperacillin/tazobactam, no MICs were above 1 mg/L. At 0.25 mg/L and 0.5 mg/L it inhibited 89.2% (83/93) and 98.9% (92/93) of isolates, respectively. All 16 β-lactamase-positive isolates were inhibited at ≤0.03 mg/L. There was some correlation between the piperacillin-tazobactam and azithromycin MICs (R=0.20, p=0.05) which became stronger when isolates with azithromycin MICs >2 mg/L (i.e. those likely to have ribosomal- rather than efflux-determined resistance) were excluded (R=0.45, p<0.001). Correlation was also observed with ceftriaxone MICs (R=0.37, p<0.001); crucially, however, all eight ceftriaxone-resistant isolates, with MICs >0.125 mg/L were inhibited by piperacillin/tazobactam at <1 mg/L, including the five isolates with high ceftriaxone MICs (>0.5 mg/L); two, with ceftriaxone MICs of 1 mg/L, were susceptible at 0.06 mg/L.

In contrast to piperacillin/tazobactam, aztreonam offered little gain compared with ceftriaxone; rather, there was a strong correlation between MICs of aztreonam and ceftriaxone (R=0.82, p<0.001), with aztreonam MICs >16 mg/L for all but one of isolates with ceftriaxone resistance. Among isolates with azithromycin MICs >0.5 mg/L there was a large range of aztreonam MICs (0.25- ≥16 mg/L) with some correlation (R=0.20, p=0.054); again, this became stronger when only azithromycin MICs ≤2 mg/L were considered (R=0.36, p<0.001).

Fosfomycin had a narrow MIC range of 8 – 64 mg/L, with 87.1% (81/93) of isolates inhibited at ≤32 mg/L (Table 1). There was no evidence of a correlation between fosfomycin MICs and those of either azithromycin or ceftriaxone.

In the case of chloramphenicol, MICs ranged from 0.5-16 mg/L, with some hint of bimodality (peaks at 1 and 4 mg/L); 79.6% (74/93) of isolates were inhibited at ≤4 mg/L and 97.8% (91/93) at ≤8 mg/L. Chloramphenicol had a wide scatter of MICs (1-16 mg/L) for isolates with azithromycin-MICs >0.5 mg/L but some correlation was observed (R=0.25, p=0.02) and this strengthened when only

isolates with azithromycin MICs ≤2 mg/L were included (R=0.40, p<0.001). Correlation with ceftriaxone was detected (*R*=0.31, *p*=0.002); thus, chloramphenicol MICs for all eight ceftriaxone-resistant isolates were in the 4-8 mg/L range.

Co-trimoxazole MICs were clustered at 8 mg/L and there was evidence of a correlation with the azithromycin MICs (R=0.3, p≤0.011). Co-trimoxazole MICs for ceftriaxone-resistant isolates were 4-8 mg/L, and those for isolates with azithromycin MICs >0.5 mg/L were consistently ≥8 mg/L.

MICs of rifampicin were bimodal, clustering around 0.25 mg/L for 52.7% (49/93) of the collection but exceeding 16 mg/L for 38.7% (36/93) (Table 1). Four of the eight ceftriaxone-resistant isolates and eight of the 20 with azithromycin MICs >0.5 mg/L were among those with low rifampicin MICs (Table 2). Interestingly, evidence of a correlation between rifampicin and azithromycin MICs (R=0.23, p=0.03) was lost when only azithromycin MICs ≤2 mg/L were compared (R=0.13, p=0.20).

Discussion

These *in vitro* studies, predominantly using multi-resistant gonococci, suggest some potential for several older agents. Extrapolation to clinical settings is complicated by two factors. First, none of these older agents has clinical breakpoints for *N. gonorrhoeae*. Secondly, MIC correlations between azithromycin (particularly for isolates with azithromycin MICs ≤2 mg/L) and piperacillin/tazobactam, chloramphenicol, co-trimoxazole and aztreonam suggest that upregulated efflux reduces susceptibility to these agents, though the MIC levels at which this has clinical impact is uncertain. Therefore, in discussing these results we have considered (i) breakpoints for other bacteria; (ii) ECOFFs for *N. gonorrhoeae* where available, and (iii) any published clinical experience, largely from old trials.

Piperacillin/tazobactam proved surprisingly active, with no MICs >1 mg/L. There was some correlation between the piperacillin/tazobactam and ceftriaxone MICs but, as with ertapenem,[12, 13]

some (not all) of the isolates with the highest ceftriaxone MICs (>0.5 mg/L) were inhibited by low concentrations of piperacillin-tazobactam. Thus piperacillin/tazobactam may present a treatment option for infection with ceftriaxone-resistant *N. gonorrhoeae*. Low piperacillin/tazobactam MICs have also been observed by others for highly-cephalosporin resistant isolates[14] and, whilst MICs rise with those of penicillin in general, they may 'top out'. There are old data for clinical use of piperacillin in gonorrhoea,[15] though dosages may need to be adjusted for more resistant isolates; tazobactam protects against β-lactamase where present. The disadvantage with piperacillin/tazobactam is the parenteral route of administration and the short half-life, meaning that multiple daily dosing is likely to be required. As a once-a-day agent, ertapenem is likely to be more convenient, where active. We did not evaluate ertapenem here as there are already many *in vitro* data available.[12-14] Generally, ertapenem has similar activity to ceftriaxone, but for some isolates with raised ceftriaxone MICs, the ertapenem MIC is lower. This has allowed some infections of extensively-drug resistant *N. gonorrhoeae* to be successfully treated with ertapenem when ceftriaxone has failed.[4, 5]

The other β-lactam tested here, aztreonam, showed no promise, with MICs for ceftriaxone-resistant isolates higher than those of ceftriaxone. It does however remain of interest in the treatment of susceptible infections in penicillin-allergic patients. A recent clinical trial found that a single dose of aztreonam 2 g IM cured 2/6 pharyngeal infections, 3/4 rectal infections and 11/11 urethral infections. All treatment failures occurred at MIC ≥0.25mg/L.[16] Similarly to our study, all of the aztreonam MICs were higher than the ceftriaxone MICs.

Several of the other agents included here were evaluated clinically in the late 1960s and 1970s as treatments for penicillin-resistant gonococcal infections. Caution must be taken when extrapolating these findings to the present day, as the *N. gonorrhoeae* population is likely to have changed over time; in particular, more isolates may have up-regulated efflux, which can affect chemically diverse agents.

Fosfomycin is perhaps the most attractive non-β-lactam, because of its narrow MIC distribution. It is a well-tolerated agent that is commonly used, as the trometamol salt, for urinary-tract infections; IV formulations are also available, achieving much higher systemic levels. A study in the 1970s showed that intramuscular fosfomycin was effective in treating gonorrhoea when multiple doses were used; 11/12 patients were cured with two doses of 2 g four hours apart, and 15/15 patients cured with 2 g every eight hours for 2 days),[17] but it was less effective (37/43 patients cured) when single dose 4 g was administered. Treatment failures were seen (17/23) with oral fosfomycin (500 mg q6h for four days).[18] In a more recent randomised controlled trial of men with uncomplicated gonococcal urethritis, fosfomycin trometamol 3 g orally was given on days 1, 3 and 5, with a reported cure rate of 96.8% (60/62 patients),[19] with this improvement likely reflecting the high dosage and the use of a better absorbed formulation. As here, recent studies evaluating fosfomycin *in vitro* against *N. gonorrhoeae* have generally found low MICs;[20] however, it is possible that resistance could emerge quite rapidly, as has been seen with *Klebsiella pneumoniae*,[21, 22] though not urinary *Escherichia coli*. Disappointingly, fosfomycin single dose 6 g orally was dropped from a recent clinical trial of new treatments for uncomplicated anogenital gonorrhoea after an interim analysis, suggesting that this fosfomycin regimen may not be clinically efficacious.[23]

Early studies evaluating co-trimoxazole used several different regimens, and cure rates varied from 66 to 100%; large doses and multi-day regimens had higher cure rates than single doses and treatment failures were associated with raised MICs.[24-27] In 1988, a study of 119 patients with pharyngeal gonorrhoea found cure rates of 97% with a five-day schedule and 89.8% with a two-day schedule.[28] Failure was seen with MICs ≥0.5 mg/L of trimethoprim and ≥9.5 mg/L sulfamethoxazole,[29] whereas cure was predictable when the isolates were inhibited by ≤0.63/11.87 mg/L of TMP/SMZ (fixed ratio, 1:19).[30] Considering the EUCAST ECOFF of 8 mg/L (with respect to sulfamethoxazole); 72/93 of the present isolates, including those with the highest-levels of

ceftriaxone resistance were inhibited, suggesting potential, though a significant minority would remain resistant if this was used as a clinical breakpoint.

There are no clinical data for chloramphenicol in gonorrhoea; however, thiamphenicol, a related molecule with a similar spectrum of activity and MICs, has been used in Africa. Unlike chloramphenicol, thiamphenicol is not associated with aplastic anaemia. Among 50 000 patients with uncomplicated gonorrhoea treated with a single 2.5 g dose between 1961 and 1982 the average failure rate was just over 3%.[31] However, a thiamphenicol modal MIC of 0.5 mg/L was reported in the African study, whereas most of our multi-resistant isolates were only inhibited at chloramphenicol concentrations around 4-8 mg/L (Table 2), perhaps indicating some temporal reduction in susceptibility for a drug that is likely to be a substrate for efflux. There is no resistance breakpoint for *N. gonorrhoeae*, but the ECOFF would be around 4 mg/L based on the EUCAST distribution.

A study of 103 patients with gonococcal urethritis treated with a single dose of 1200 mg rifampicin found a 91% cure rate; 3/3 patients with pharyngeal infection were also cured.[32] Trials in the 1980s also found that a combination of rifampicin plus erythromycin was effective.[33] In our study the bimodal MIC distribution suggests two populations; wild type and non-wild type, with the latter likely to harbour acquired resistance mutations. Given the old clinical data, the drug may be of use where otherwise multi-resistant isolates remain susceptible *in vitro*, though the incidence of emerging resistance would require exploration. Rifampicin resistance readily arises in many organisms through a single point mutation and has been shown to emerge in *N gonorrhoeae* previously.[34, 35] This would need to be considered in the use of rifampicin as part of a treatment regimen for gonorrhoea, and almost certainly excludes its use as monotherapy.

Our study does not suggest that any of the agents studied could be included as part of national empirical treatment guidelines, either alone or in combination, particularly as clinical

breakpoints for these agents have not been defined. However, several –piperacillin/tazobactam, fosfomycin, co-trimoxazole, rifampicin and, possibly, chloramphenicol– might be considered as part of a pragmatic approach when treating individual patients with resistant infection, once MICs are available. Aztreonam, as well as the non-β-lactam agents, may be useful for susceptible infections in patients with severe penicillin allergy. For cases with infection caused by *N. gonorrhoeae* isolates with reduced susceptibility to ceftriaxone, a possible treatment strategy could be to combine high-dose ceftriaxone plus one of the non-β-lactam agents, although susceptibility testing would be needed to determine the best choice. A recent *in vitro* study of combinations of ceftriaxone or cefixime with rifampicin or fosfomycin found that no combinations were antagonistic nor synergistic.[36] However, there are no clinical data to support the use of these combinations.

Wider surveillance of the susceptibility of *N. gonorrhoeae* to these agents is needed, as well as clinical trials to define susceptibility breakpoints and determine the effectiveness of these agents in treating gonococcal infection at both genital and extra-genital sites.

Acknowledgments

We would like to thank the GRASP team and the GRASP Collaborators for submitting isolates.

Funding

This study was funded by Public Health England.

Transparency declarations:

PHE's Antimicrobial Resistance and Healthcare Associated Infections Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including Accelerate Diagnostics; Achaogen, Inc.; Allecra Therapeutics; Amplex; AstraZeneca UK, Ltd.; AusDiagnostics; Basilea Pharmaceutica; Becton, Dickinson

Diagnostics; bioMérieux; Bio-Rad Laboratories; The BSAC; Cepheid; Check-Points B.V.; Cubist Pharmaceuticals; Department of Health; Enigma Diagnostics; European Centre for Disease Prevention and Control; Food Standards Agency; GlaxoSmithKline Services, Ltd.; Helperby Therapeutics; Henry Stewart Talks; IHMA, Ltd.; Innovate UK; Kalidex Pharmaceuticals; Melinta Therapeutics; Merck Sharpe & Dohme Corp.; Meiji Seika Pharma Co., Ltd.; Mobidiag; Momentum Biosciences, Ltd.; Neem Biotech; NIHR; Nordic Pharma, Ltd.; Norgine Pharmaceuticals; Rempex Pharmaceuticals, Ltd.; Roche, Rokitan, Ltd.; Smith & Nephew UK, Ltd.; Shionogi & Co., Ltd.; Trius Therapeutics; VenatoRx Pharmaceuticals; Wockhardt, Ltd.; and the World Health Organization.

DML: Advisory Boards or ad-hoc consultancy Accelerate, Allecra, Antabio, Centauri, Entasis, GlaxoSmithKline, Meiji, Melinta, Menarini, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, T.A.Z., Tetraphase, VenatoRx, Wockhardt, Zambon, Paid lectures – Astellas, bioMérieux, Beckman Coulter, Cardiome, Cepheid, Merck/MSD, Menarini, Nordic, Pfizer and Shionogi. Relevant shareholdings or options – Dechra, GSK, Merck, Perkin Elmer, Pfizer, T.A.Z, amounting to <10% of portfolio value.

HF, TU, NW, MC: none to declare

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Table 1. MIC distribution for 93 *N. gonorrhoeae* isolates

Antimicrobial	**Number of isolates with MIC (mg/L)**																
	≤0.004	**0.008**	**≤0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**>16***	**32**	**64**	**>256**
Fosfomycin												4	45		32	12	
Piperacillin/tazo-bactam			50	10	7	6	10	9	1								
Chloramphenicol								11	22	7	34	17	2				
Co-trimoxazole **										4	11	57	13	8			
Aztreonam						2	9	29	20	4	13	7	1	8			
Rifampicin					5	9	30	10		1			2	36			
Azithromycin					10	16	26	21	4	3	4	3†	2†				4†
Ceftriaxone	3	15	28	18	17	4	2	1†	4†	1†							

*Includes isolates at the end of the agar dilution scale with MICs >16 mg/L

**Expressed relative to trimethoprim

†MIC determined by Etest

Table 2. MICs for 27 *N. gonorrhoeae* isolates with ceftriaxone MICs >0.125 mg/L and/or azithromycin MICs >0.5 mg/L, sorted by ceftriaxone MIC (descending)

Isolate	CRO	AZM	AZT	CHL	SXT	FOS	TZP	RIF
WHO X	2	0.25	>16	8	8	16	0.5	0.25
19NG15	1	>256	>16	4	8	16	0.06	16
19NG17	1	0.5	>16	8	8	32	0.5	>16
19NG16	1	0.5	>16	8	8	16	0.25	>16
WHO Y	1	0.5	>16	8	8	64	0.06	0.25
19NG18	0.5	0.125	>16	4	8	16	0.25	>16
WHO Z	0.25	0.5	>16	8	8	16	0.25	0.5
WHO L	0.25	0.25	2	4	4	16	1	0.25
RB528	0.125	1	8	8	8	32	0.5	0.5
19NG11	0.06	>256	2	4	>16	32	0.03	>16
RB1999	0.06	2	1	1	>16	16	<=0.015	>16
RB2261	0.06	1	8	8	8	16	0.5	0.25
QA15-10	0.06	1	4	8	8	32	0.5	0.25
19NG12	0.03	>256	1	4	8	16	0.125	>16
WHO V	0.03	>256	1	2	>16	64	0.03	0.25
19NG02	0.03	12	4	16	8	16	0.06	0.25
19NG08	0.03	12	1	1	8	16	0.03	0.125
RB154	0.015	8	2	16	8	16	0.25	>16
WHO P	0.015	4	0.5	1	8	64	<=0.015	>32
19NG09	0.015	4	1	1	>16	64	<=0.015	>16
RB2378	0.015	4	0.5	4	8	32	0.03	0.125
19NG00	0.015	2	0.5	1	16	32	<=0.015	>16
19NG03	0.015	2	0.5	1	16	16	<=0.015	>16
QA15-07	0.015	1	4	4	>16	32	0.06	0.5
19NG10	0.008	8	1	1	16	32	<=0.015	>16

19NG06	0.008	8	1	1	8	32	<=0.015	>16
WHO U	0.008	4	0.25	4	8	32	<=0.015	>16

369 Note: WHO V β-lactamase positive

370 CRO – ceftriaxone, AZM – azithromycin, AZT – aztreonam, CHL - chloramphenicol, SXT-
371 trimethoprim/sulphamethoxazole (co-trimoxazole), FOS – fosfomycin, TZP - piperacillin/tazobactam,
372 RIF - rifampicin

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