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Original Article

Broad-Spectrum Antibiotics are Associated with worse Survival After Radical Treatment for Glioblastoma Multiforme: A Multicentre Study

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Abstract

Aims: Glioblastoma (GBM) is the most common and aggressive primary brain malignancy in adults. Antibiotic exposure is associated with decreased survival in several solid cancers, but its impact in GBM has not been investigated. We assessed the effect of nonprophylactic antibiotic exposure on survival in patients with GBM receiving radical treatment.

Materials and methods: This retrospective cohort study analysed patients with the World Health Organization (WHO) Grade 4 isocitrate dehydrogenase (IDH)-wildtype GBM treated with radiotherapy, with or without concurrent and/or adjuvant temozolomide. Overall survival (OS) was the primary outcome. Antibiotic exposures were recorded from four weeks before radiotherapy until completion of adjuvant chemotherapy, excluding peri-operative, prophylactic and peri-mortem use.

Results: In the discovery cohort ($N = 234$), antibiotic exposure was associated with significantly reduced OS ($P = 0.0062$). Multivariate and sensitivity analyses (HR = 1.46, $P = 0.045$), controlling for confounders (eg, corticosteroid exposure, infection occurrence and timing) supported the association. Findings were reproduced in an independent validation cohort ($N = 139$). Exploratory unpowered subgroup analyses suggest that the adverse effect of antibiotics was most pronounced in patients with a better prognosis.

Conclusion: Antibiotic exposure during GBM treatment may be associated with reduced survival. Further research evaluating the role of the microbiome in GBM treatment outcomes is warranted. Our results support judicious antibiotic stewardship to maximise therapeutic outcomes.

Introduction

Glioblastoma (GBM) is the most common adult primary brain malignancy, affecting approximately 300,000 patients per year globally and has a rising incidence [1,2]. It is a major cause of cancer-related mortality and morbidity in adults under 40 and has a rising incidence [3]. GBM treatment has not changed significantly since the Stupp chemoradiotherapy protocol nearly twenty years ago [4]. Optimal treatment includes maximal safe surgical resection followed by chemoradiotherapy and adjuvant chemotherapy with temozolomide [5]. However, GBM consistently recurs, and the median survival with treatment remains less than 18 months [4,5].

Radiotherapy and chemotherapy, which are key modalities in GBM treatment, are associated with a number of toxicities that may require antibiotic therapy. The majority of antibiotics prescribed are broad-spectrum and alter the normal balance of bacterial communities. The Stupp

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protocol routinely includes the prescription of low-dose prophylactic antibiotics to prevent complications such as *Pneumocystis jirovecii* pneumonia (PJP). However, increasing evidence suggests that the administration of antibiotics impacts cancer treatment efficacy, and clinical observations show an association between antibiotic exposure and reduced survival, for example, in patients treated with radiotherapy or immunotherapy. [6,7]. No published studies have assessed whether antibiotics impact the efficacy of GBM treatment.

Here, we explore whether antibiotic therapy during treatment for WHO grade 4 IDH-wildtype GBM affects patient survival outcomes, with findings validated across two independent cohorts. Our hypothesis-generating findings suggest that the microbiome may impact GBM treatment efficacy.

Materials and Methods

Data (Supplementary Table 1) were collected retrospectively from electronic patient records at two tertiary neuro-oncology centres. The study received Institutional Review Board approval, and written informed consent was obtained from all patients according to the Declaration of Helsinki.

Eligible patients had WHO Grade 4 IDH-wildtype glioblastoma and underwent radiotherapy with either 40 Gy in 15 fractions over three weeks or 54–60 Gy in 30 fractions over six weeks. Treatment included concurrent and/or adjuvant temozolomide chemotherapy: 75 mg/m^2 daily during radiotherapy (CC) and 150–200 mg/m^2 for up to six cycles post-radiotherapy (AC). Patients not completing planned radiotherapy were excluded. Where delivered, prophylactic co-trimoxazole (960 mg BD, given thrice weekly) was administered to patients receiving chemoradiotherapy.

Antibiotic exposures were defined as any antibiotic prescribed during treatment not limited to those prescribed for infective episodes. Duration of antibiotic exposure was limited to a single prescription period of 5–7 days from initiation. Repeat prescriptions were counted as a separate antibiotic exposure. Antibiotic exposures were documented from four weeks before radiotherapy to the end of AC, excluding exposures within 30 days of death, perioperative and prophylactic exposures. Perioperative antibiotics were excluded from analysis as these were received by all patients as part of perioperative management in both cohorts. Prophylactic co-trimoxazole antibiotics were excluded, except in exploratory analysis, as this is part of standard treatment in patients undergoing chemoradiotherapy for glioblastoma as per the Stupp protocol [8]. Patients who died due to infective complications were excluded. Infections were recorded as pre-radiotherapy (excluding perioperative), during radiotherapy, during AC, or as re-infections ($\geq$2 infections in different treatment periods). Surgical resection extent was classified as biopsy, sub-total, or gross-total based on postoperative imaging reviewed in tumour board meetings. Corticosteroid use was quantified as total milligrams of dexamethasone equivalence over the periods of four weeks before, during, and four weeks after radiotherapy. Patients were categorised into above- or below-median cohort corticosteroid use as per previously published approaches [9]. Overall survival (OS) was measured from the start of radiotherapy to death.

Statistical Considerations

This exploratory retrospective study was based on a fixed number of eligible patients treated during the study period. Because the presence and magnitude of the observed effect were unknown *a priori*, a formal sample size calculation was not possible.

Analyses were conducted using R version 4.3.2.8 Patient characteristics were presented as means and frequencies. The chi-squared test assessed statistical significance. The impact of antibiotic exposures on survival was evaluated using the Kaplan-Meier methodology. Cox proportional hazards regression was performed with the coxph(.) function in the survminer R package. Patients were stratified by number of antibiotic courses (0 vs 1 vs $\geq$2) where each group met the prespecified criterion of a representing $\geq$15% of the cohort; otherwise, patients in the antibiotic group were grouped together.

Sensitivity analysis was performed to ensure the robustness of the model. The proportional hazards assumption was first assessed using Schoenfeld residuals, and variables that violated this assumption ($P \leq 0.05$) were excluded from further analysis. The refined model was then subjected to bootstrap resampling with 1000 iterations to validate the stability and reliability of the remaining model coefficients.

Patients without documented OS events were censored at the last available information date. All tests were two-sided, with significance defined as $P \leq 0.05$.

Results

Demographics – Discovery Cohort

The discovery cohort included 234 patients (Table 1) treated for GBM between May 2016 and March 2022 at a tertiary centre in the UK who were available for analysis.

The majority of patients had a performance status (PS) $\leq$ 1 (83%) and the mean Charlson Comorbidity Index (CCI) was 1.85 (standard deviation [SD] 1.23), reflecting limited comorbidities across the cohort. There were no differences in PS or comorbidity burden between groups. The reasons for antibiotic prescription were primarily respiratory infections (40%). The majority of antibiotics prescribed were penicillins (61%), followed by tetracyclines (8%). All patients treated with concurrent chemotherapy received prophylactic low-dose cotrimoxazole (78%) in line with international protocols. There was a slight (54.81% vs 50.51%) but statistically significant lower proportion of patients who had gross-total resection in the antibiotic group ($P = 0.019$).

Table 1
Patient characteristics by antibiotic treatment − discovery cohort

Characteristics	Total	Antibiotics		*P* value
	(N = 234)	No	Yes	
		(N = 135)	(N = 99)	
Demographics				
Gender				
Male	149 (63.68)	81 (60.00)	68 (68.69)	
Female	85 (36.36)	54 (40.00)	31 (31.31)	
Median age in years (IQR)	60 (52−68)	60 (51−66)	61 (53−68)	$P = 0.17$
Performance status				
PS 0-1	194 (82.91)	112 (82.96)	82 (82.83)	$P = 0.98$
PS ≥2	40 (17.09)	23 (17.04)	17 (17.17)	
Charlson Comorbidity Index				
CCI 0-1	98 (41.88)	62 (45.93)	36 (36.36)	$P = 0.18$
CCI ≥2	136 (58.12)	73 (54.07)	63 (63.64)	
Tumour and treatment characteristics				
Extent of resection				
Biopsy	75 (32.05)	35 (25.93)	40 (40.40)	$P = 0.02$*
Subtotal resection	124 (52.99)	74 (54.81)	50 (50.51)	
Gross-total resection	35 (14.96)	26 (19.26)	9 (9.09)	
MGMT promoter methylation status				
MGMT promoter methylated (>10%)	96 (41.03)	53 (39.36)	43 (43.43)	$P = 0.52$
MGMT promoter unmethylated (<10%)	138 (58.97)	82 (60.74)	56 (56.57)	
Radiotherapy dose				
40 Gy	61 (26.07)	33 (24.44)	28 (28.28)	$P = 0.51$
54−60 Gy	173 (73.93)	102 (75.56)	71 (71.72)	
Concurrent chemotherapy (CC)				
Yes	182 (77.78)	102 (75.56)	80 (80.81)	$P = 0.34$
No	52 (22.22)	33 (24.44)	19 (19.19)	
Adjuvant chemotherapy (AC)				
Yes	155 (66.24)	91 (67.41)	64 (64.65)	$P = 0.66$
No	79 (33.76)	44 (32.59)	35 (35.35)	
Mean number of cycles (±SD) [All]		3.45 (2.63)	2.96 (2.63)	$P = 0.16$
Mean number of cycles (±SD) [MGMT promoter methylated +60 Gy + CC]		4.35 (2.63)	3.33 (2.63)	$P = 0.11$
Mean total cumulative corticosteroid (dexamethasone) dose (mg) received during RT (SD)	306.50 (195.67)	297.66 (201.03)	318.57 (188.47)	$P = 0.28$
Mean total cumulative corticosteroid (dexamethasone) dose (mg) received during entire course of treatment (SD)	484.68 (317.63)	490.44 (349.27)	463.75 (271.83)	$P = 0.73$
Reasons for antibiotic prescription				
Respiratory infection	40%	-	-	
Urinary infection	15%	-	-	
Skin infection	9%	-	-	
Wound infection	7%	-	-	
Ear/eye infection	6%	-	-	
Dental infection	3%	-	-	
Gastrointestinal infection	3%	-	-	
Salivary duct infection	1%	-	-	
Intracranial infection	1%	-	-	
Not documented	15%	-	-	

Values are n (%) unless otherwise indicated. IQR, interquartile range; CCI, Charlson Comorbidity Index; PS, performance status; SD, standard deviation.

There were no reported cases of PJP. Only 6 patients had postoperative infections and, in an exploratory analysis, we did not find any significant relationships with survival ($P = 0.250$). All patients received corticosteroids at some stage during their treatment, though dosage varied. Median total corticosteroid exposure was 448 mg dexamethasone equivalent during the whole treatment (radiotherapy with or without concurrent and/or adjuvant chemotherapy) and 252 mg (interquartile range [IQR]: 168 mg–399 mg) during radiotherapy. No significant differences were found in corticosteroid dose between groups either during the whole treatment ($P = 0.419$) or radiotherapy (0.726).

Median follow-up was 13 months (2–79 months). Overall, 204 patients died during the follow-up period. Median OS was 13.5 months (95% confidence intervals [CIs]:12.2–15.3). The large majority of deaths (98%) were attributable to cancer progression. No patients died of infection-related complications, as this was an exclusion criterion. Any antibiotics taken perimortem were not considered for stratifying groups.

Demographics – Validation Cohort

To test the reproducibility of our findings, we collected data from an independent cohort treated at a different tertiary centre in the UK (Table 2). This validation cohort, comprised 139 patients with IDH-wildtype GBM treated between May 2016 and March 2022, all treated with 54–60 Gy in 30 fractions over six weeks. Median follow-up was 15.7 months (2–89 months) and 123 patients died. Median OS

Table 2
Patient characteristics by antibiotic treatment – validation cohort

Characteristics	Total	Antibiotics		*P* value
	(N = 139)	No	Yes	
		(N = 95)	(N = 44)	
Demographics				
Gender				
Male	97 (69.78)	69 (72.63)	28 (63.64)	$P = 0.28$
Female	42 (30.22)	26 (27.37)	16 (36.36)	
Median age, years (IQR)	60 (53–64)	60 (53–65)	59.5 (54–64)	
Performance status				
PS 0-1	131 (94.24)	89 (93.68)	42 (95.45)	$P = 0.68$
PS ≥2	8 (5.76)	6 (6.32)	2 (4.55)	
Charlson Comorbidity Index				
CCI 0-1	57 (41)	38 (40)	19 (43)	$P = 0.16$
CCI ≥2	82 (59)	57 (60)	25 (57)	
Tumour and treatment characteristics				
Extent of resection				
Biopsy	31 (22.30))	21 (22.10)	10 (22.73)	$P = 0.64$
Subtotal resection	91 (65.47)	64 (67.37)	27 (61.36)	
Gross-total resection	17 (12.23)	10 (10.53)	7 (15.91)	
MGMT promoter methylation status				
MGMT promoter methylated (>10%)	52 (37.41)	36 (37.89)	16 (36.26)	$P = 0.86$
MGMT promoter unmethylated (<10%)	87 (62.59)	59 (62.11)	28 (63.64)	
Concurrent chemotherapy (CC)				
Yes	131 (94.24)	91 (95.79)	40 (90.91)	$P = 0.25$
No	8 (5.76)	4 (4.21)	4 (9.09)	
Adjuvant chemotherapy (AC)				
Yes	98 (70.50)	72 (75.79)	24 (54.55)	$P = 0.012$*
No	41 (29.50)	23 (24.21)	20 (45.45)	
Mean number of cycles (±SD) [All]		3.07 (2.56)	2.55 (2.55)	$P = 0.27$
Mean number of cycles (±) [MGMT promoter methylated +60 Gy + CC]		3.29 (2.55)	2.25 (2.55)	$P = 0.17$
Mean total cumulative corticosteroid (dexamethasone) dose (mg) received during RT (SD)	228.38 (152.45)	221.75 (152.58)	242.69 (152.94)	$P = 0.38$

Values are n (%) unless otherwise indicated. IQR, interquartile range; CCI, Charlson Comorbidity Index; PS, performance status; SD, standard deviation.

was 15.70 months (95% CI: 13.03–19.00). The majority of patients had PS 0–1 (94%) and mean CCI was 1.71 (SD: 1.00), reflecting limited comorbidities. There were no significant differences between groups except for AC, which was more represented in the group that did not receive antibiotics (75.79% vs 54.55%; $P = 0.012$).

About a third of patients received antibiotics during their oncological therapy, mostly for respiratory (37%) or urinary (22%) infections. The majority of antibiotics prescribed were penicillins (49%), followed by tetracyclines (9%). Fourteen patients (10%) received ≥2 courses of antibiotics. Due to a change in local protocols during this period, 84 (60%) patients did not receive prophylactic cotrimoxazole during their treatment. Median total corticosteroid exposure during the complete treatment period (concurrent chemo-radiotherapy and adjuvant chemotherapy) was 196 mg (IQR: 98 mg–336 mg) dexamethasone equivalent and there were no differences in corticosteroid exposure between groups ($P = 0.380$). Additionally, corticosteroid use was not associated with antibiotic use ($P = 0.567$). As with the discovery cohort, there were no reported cases of PJP. Again, antibiotics were highly associated with diagnosed infections ($P < 0.001$).

Antibiotic Exposures are Associated with Reduced Survival

In the discovery cohort, survival was significantly lower in patients who had received antibiotics compared to those who had not (12.03 vs 15.23 months; $P = 6.2.10^{-3}$; Figure 1A).

To analyse the impact of antibiotic uptake while controlling for potential confounders, we firstly defined prognostic predictors through a review of the literature research. Established predictors of prognosis included PS, corticosteroid uptake, extent of resection (biopsy vs subtotal vs gross total resection), concurrent chemotherapy, adjuvant chemotherapy, radiotherapy interruption >2 days, radiation dose (40 Gy in 15 fractions or 54–60 Gy in 30 fractions), O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status and Charlson Comorbidity Index [8,10–12]. We then used univariate analysis to detect which of these predictors were significantly associated with OS in this cohort (Supplementary Table 2). All predictors except radiotherapy interruptions were significantly associated with OS and thus were carried forword to multivariate analysis (MVA).

Separately, we analysed associations between infections and survival, as the impact of the infections themselves rather than antibiotic uptake may determine efficacy outcomes. Antibiotic uptake was strongly associated with infections as expected ($P < 0.0001$). Kaplan-Meier modelling (Supplementary Figure 1), showed a significant association between timing of infection and survival ($P = 0.021$). However, pairwise comparisons showed a significant survival difference only between the *no infections* vs *infection during radiotherapy* groups ($P = 0.026$). Importantly, no significant differences were found when comparing the *no infections* and *re-infection* groups ($P = 0.285$). As such, infection during radiotherapy was retained for MVA.

In multivariate Cox regression, antibiotic (HR: 1.46; 95% CI: 1.01–2.12, $P = 0.045$), and corticosteroid ($P < 0.001$) exposures were associated with worse survival. Extent of resection ($P = 0.002$), MGMT promoter methylation and AC (both $P < 0.001$) were associated with better survival (Figure 1B). Interestingly, the effect of infection during radiotherapy was not significant in this model ($P = 0.255$).

To test the validity of these observations, we firstly performed a sensitivity analysis (Supplementary Table 3). The proportional hazards assumption of a parsimonious Cox model, including variables that remained prognostically relevant and satisfied proportional hazards assumptions was initially tested using Schoenfeld residuals, with variables iteratively removed until an overall model where the effect of all variables and the overall model were consistent over time (ie, $P > 0.05$) was achieved. The effect of antibiotic uptake ($P = 0.200$), extent of resection ($P = 0.490$) and MGMT promoter methylation ($P = 0.420$) were retained. Subsequently, bootstrap resampling with 1000 iterations was performed on the adjusted model to confirm model coefficient stability, demonstrating that patients receiving antibiotics had a statistically significant HR of 1.218 (95% CI: 1.013–1.467), indicating ~22% higher hazard of death.

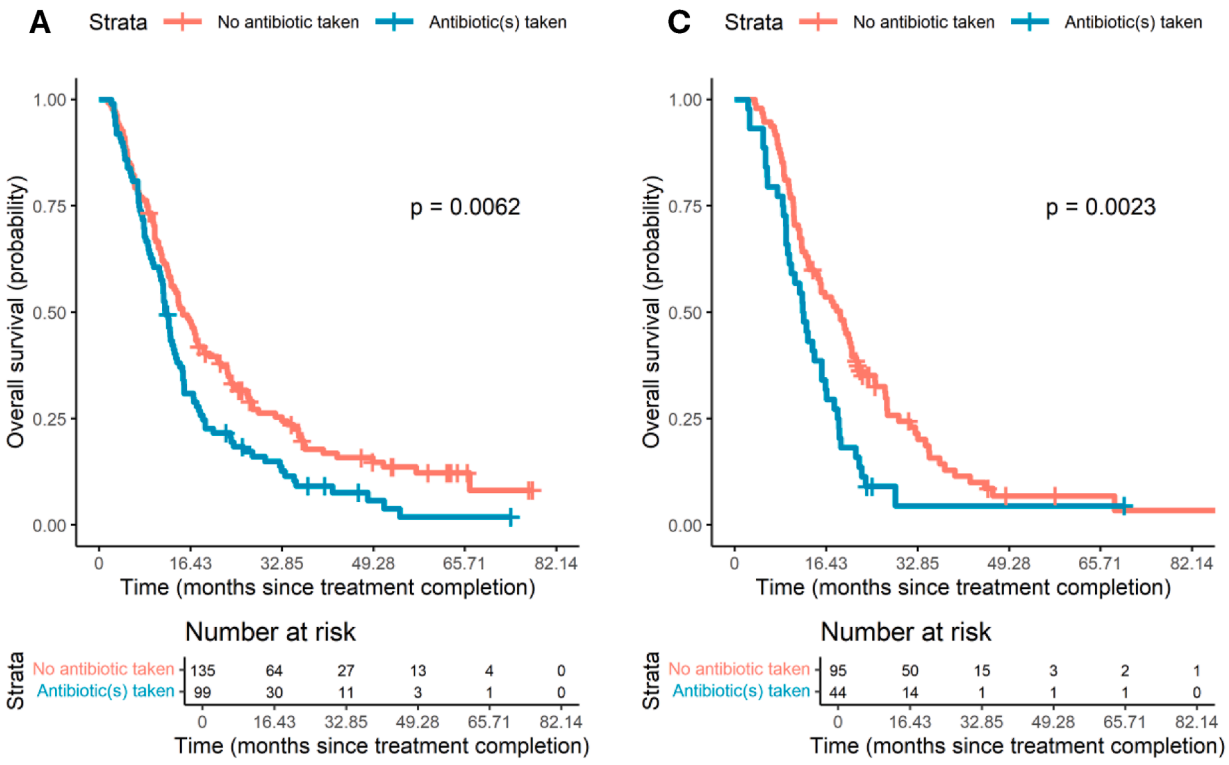
We next validated our observations in the external validation cohort. Kaplan-Meier modelling showed that antibiotic exposure was associated with reduced OS (12.46 vs 19.17 months, $P = 0.0023$, Figure 1C), consistent with discovery cohort findings.

Exploratory Analysis Suggests that Antibiotic Exposures May be More Impactful in the Fittest Patients with Best Predicted Prognosis

Although antibiotic uptake impacted on survival, we questioned whether these effects were due to compromised patient fitness or prognosis. To address this question, we performed an unplanned exploratory analysis to examine the effect of antibiotics in the subgroup of fittest patients with the better predicted prognosis, defined as patients with PS 0–1, MGMT promoter methylated tumours receiving 54–60 Gy in 30 fractions with concurrent chemotherapy ($N = 61$). Antibiotic exposure was associated with significantly reduced OS (14.5 vs 27.5 months, $P = 0.014$, Figure 2A). We did not find significant survival differences in patients stratified by infection timing (Supplementary Figure 2), which mitigates potential effects of infection rather than antibiotics on OS. In the validation cohort, where we explored a subgroup with the same characteristics ($N = 46$) we observed a similar association (9.2 vs 27.3 months, $P = 0.004$, Figure 2B). These findings suggest that antibiotic uptake may more negatively impact the fittest patients with the best predicted prognosis, who are least susceptible to infection-related complications.

Exploratory Analysis Suggests that Prophylactic Antibiotics May Also Modestly Impact Survival

Given that 60% of the validation cohort, which only included patients treated with concurrent



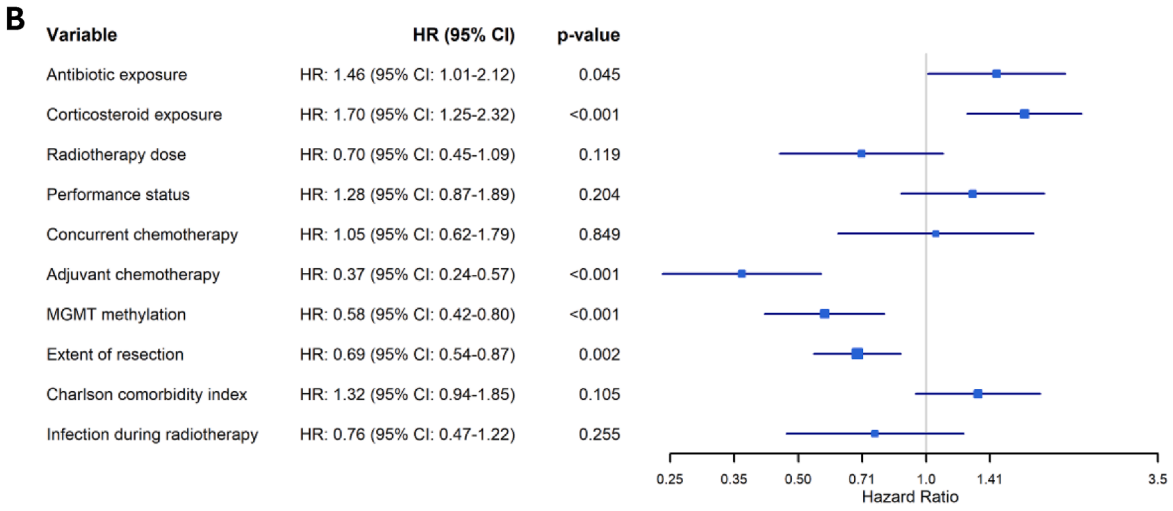


Fig. 1. Antibiotic exposure during oncological treatment for GBM is associated with worse survival. (A) Patients with GBM in the discovery cohort ($N = 234$) exposed to antibiotics during oncological treatment for GBM had worse survival. **(B)** Multivariate analysis shows that antibiotic exposures were independently significant with respect to other predictors of survival. **(C)** The negative association of antibiotic uptake with survival was also observed in the independent validation cohort ($N = 139$).
Largest in those with MGMT promoter methylated tumours receiving chemoradiotherapy (**D**). Again, this is resilient on multivariate analysis with established predictors of survival (**E**). GBM, glioblastoma.

chemoradiotherapy, was not treated with prophylactic antibiotics, we explored their impact on survival. Kaplan-Meier analysis revealed no significant associations between antibiotic prophylaxis and survival duration ($P = 0.95$, Figure 3A). We also did not find significant differences in the number of courses of nonprophylactic antibiotics taken between groups stratified by prophylactic antibiotic uptake ($P = 0.11$). However, we further questioned whether additional broad-spectrum antibiotics might bias results and to address this issue, we restratified this cohort. We found a nonsignificant ($P = 0.11$) but biologically plausible trend, where patients with neither, either or both prophylactic cotrimoxazole and antibiotics had, respectively, 19, 15, and 10 months of OS (Figure 3B). Given the limited power, these analyses should be interpreted as exploratory and are presented to illustrate potential patterns rather than to

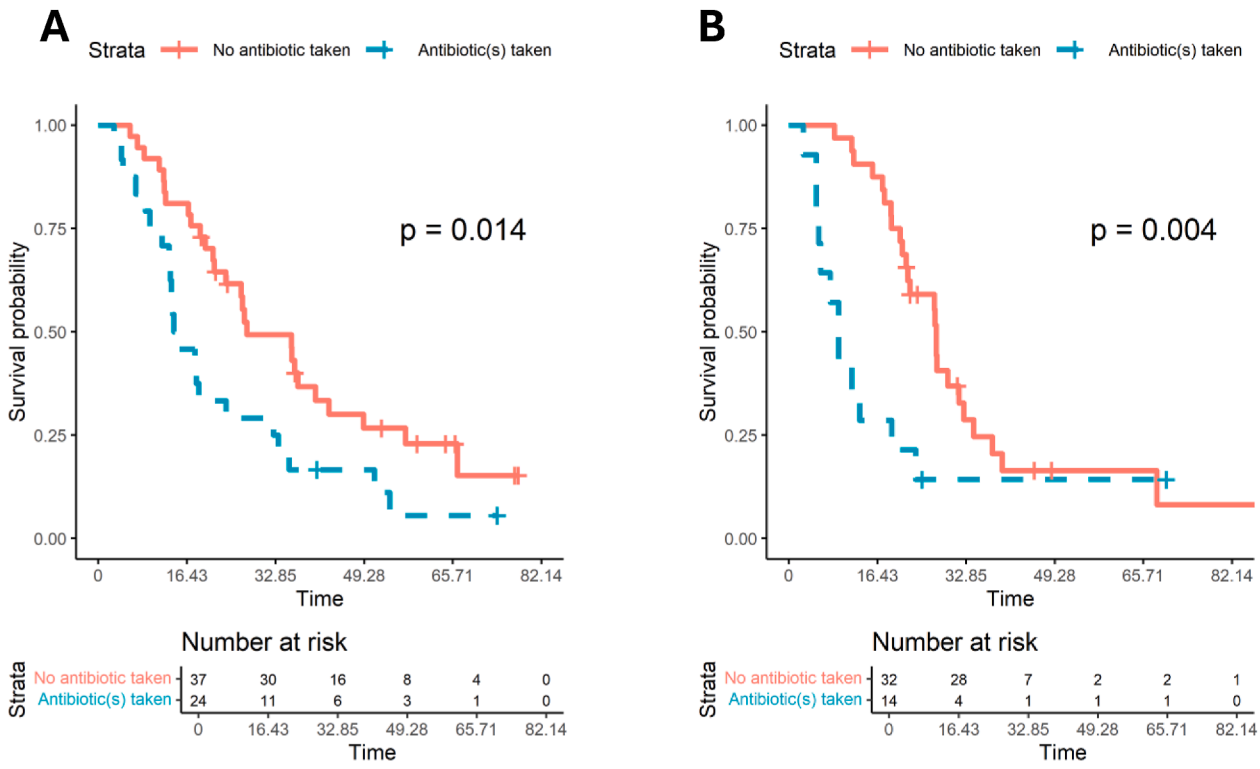


Fig 2. Exploratory subgroup analysis suggests that antibiotic exposure during GBM treatment may be associated with more significant effects in patients with better fitness (PS 0–1) and the best predicted prognosis (treated with high-dose radiotherapy with concurrent chemotherapy followed by adjuvant chemotherapy). (**A**). Discovery cohort subgroup ($N = 61$) (**B**) Validation cohort subgroup ($N = 46$). GBM, glioblastoma; PS, performance status.

support definitive inference. Results suggest that prophylactic low-dose cotrimoxazole may affect survival, but that this effect is likely modest.

Exploratory Analysis Suggests Antibiotic Route of Administration May Influence Survival

Because the number of hospital admissions and length-of-stay data were not available with sufficient accuracy, we performed an exploratory analysis to test whether the route of antibiotic administration influenced survival, using intravenous versus oral therapy as a surrogate for greater versus lesser infection severity. In the discovery cohort, 76 patients received oral antibiotics, 18 patients received intravenous antibiotics and in 5 patients the route was unknown. Median OS was shorter in the intravenous than the oral group (8.03 vs 12.67 months), although this difference was not statistically significant ($P = 0.15$). In the validation cohort, 20 patients received oral and 10 intravenous antibiotics; and the route was unknown in 5. Again, median OS was shorter in the intravenous group than in the oral group (10.87 vs 12.83 months), but this difference was not statistically significant ($P = 0.20$). These exploratory findings show a consistent directional trend across both cohorts, but do not provide definitive evidence that the route of administration significantly influenced survival. However, we note the limited power to detect these differences given the small sample size in each cohort.

Discussion

This study suggests that nonprophylactic antibiotic exposures during oncological treatment are associated with reduced survival in GBM. Exploratory analyses suggest that the impact of antibiotic uptake may be more significant in the fittest patients with the best predicted prognosis and also that prophylactic antibiotics may also modestly compromise survival.

While we stress that our study is associative and hypothesis-generating rather than definitive, our findings suggest that this association is robust and independent of other factors, including those related to treatment delivery, MGMT methylation status, fitness, comorbidities, or corticosteroid uptake.

Our analytical strategy mitigates confounding by infection-related morbidity or mortality. All patients died due to GBM-related complications and no patients died from infections. We excluded any antibiotic exposures in the perioperative period and within 4 weeks of death. Both our cohorts had a low comorbidity burden and good fitness. Additionally, our analyses of subgroups with good fitness and the best predicted prognosis showed a more profound effect on survival. All these strategies reduce the likelihood that the compromise in survival is due to either low fitness prior to treatment, its compromise following infections, significant comorbidity prior to treatment, or death from infective complications. We further performed

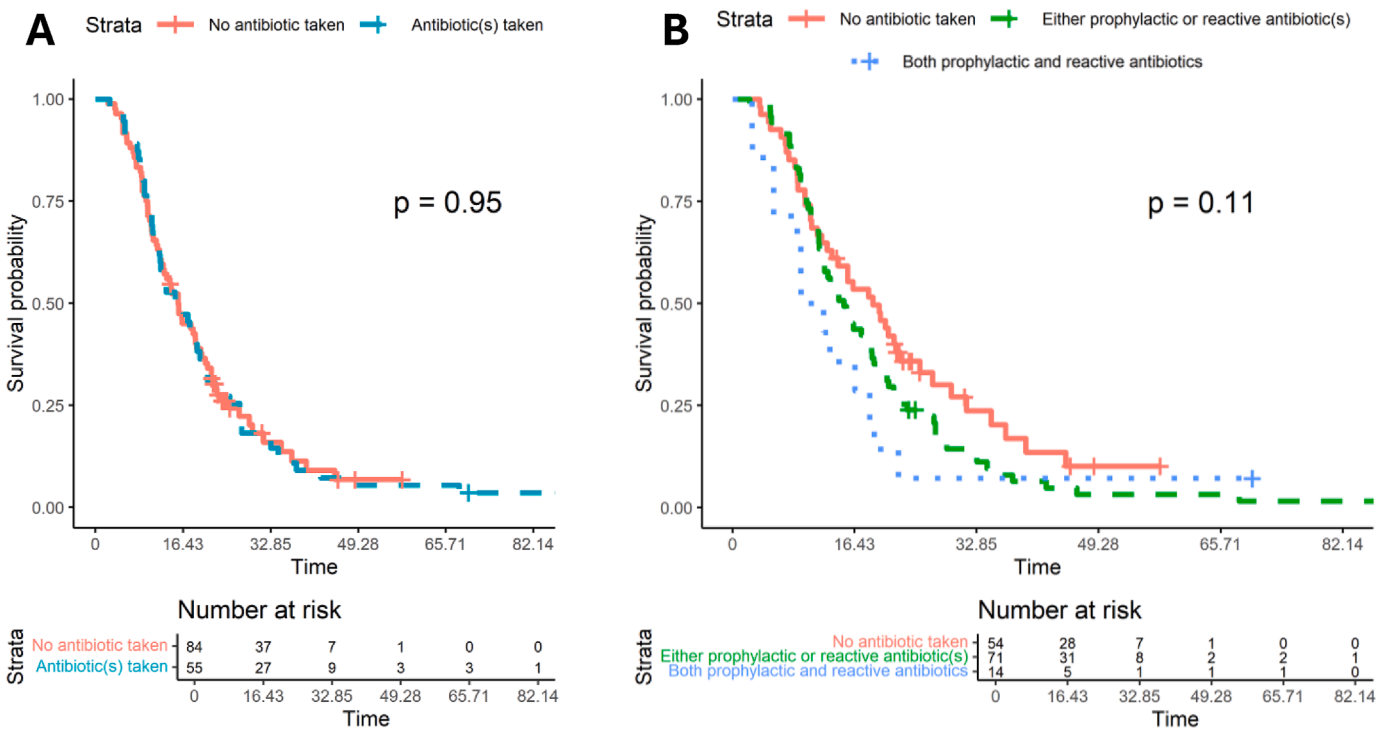


Fig 3. Hypothesis-generating analysis of prophylactic versus nonprophylactic antibiotic exposure in the validation cohort suggests that prophylactic cotrimoxazole may impact survival, but that the impact is likely modest. In the validation cohort, 55 patients received prophylactic cotrimoxazole during concurrent and/or adjuvant chemotherapy and 84 did not. (**A)** Analysis of the whole cohort stratified according to prophylactic co-trimoxazole uptake. (**B)** However, analysis of patients stratified by prophylactic and reactive antibiotics taken suggests a trend where patients exposed to neither antibiotic have better survival than patients exposed to either prophylactic or reactive antibiotics, with patients exposed to both having the worst survival outcomes.

an exploratory analysis to evaluate the route of antibiotic therapy (oral versus parenteral) as a surrogate for infection severity, showing a consistent trend towards worse OS in the intravenous antibiotic group in both cohorts, although these differences did not reach statistical significance. These findings may indicate that infection severity or differences in antibiotic administration or broader-spectrum antibiotic exposure may impact survival. However, our subgroup sample sizes were small and results should be interpreted with caution. We recognise that future prospective studies should record data including comorbidities, treatment-related toxicities, number of hospitalisations per documented infection, infection severity (sepsis and intensive care requirements), immune status (eg, white blood cell counts), which were not available for a more comprehensive characterisation of our cohorts in this retrospective study. Additionally, the effect of perioperative antibiotics could not be evaluated because they were universally administered to all patients. However, we conducted a separate exploratory analysis of prophylactic cotrimoxazole, which allowed us to consider whether different forms of antibiotic exposure contribute to the observed association with survival.

Whilst previous studies have evaluated the impact of antibiotic exposure in haematological and some solid-tissue malignancies, there is no published literature on the impact of antibiotics in primary brain tumours. Several studies have consistently suggested that treatment with antibiotics has a negative impact on cancer treatment outcomes [13–16]. Pertinently, three large studies report that antibiotic exposures compromise survival in patients treated with radical radiotherapy for head and neck and lung cancers [14,15,17]. While it is possible that the use of antibiotics correlates with toxicity-related complications of treatment and/or general frailty, the reproducibility of results across different cancers, the external validity of our observations and our statistical methodology suggest that antibiotic uptake does affect survival in patients with GBM as in other solid cancers.

Cotrimoxazole is routinely prescribed as a prophylactic antibiotic during chemoradiotherapy treatment for GBM [4]. Our exploratory analysis in the validation cohort shows that PJP prophylaxis was not significantly associated with worse survival, unlike the addition of broad-spectrum antibiotics. On the other hand, we demonstrate a biologically plausible trend where patients not treated with any antibiotics had better survival compared to patients treated with either prophylaxis or reactive antibiotics, who in turn had better survival compared to patients treated with both. These results should be interpreted with caution, but it can be suggested that prophylactic antibiotics may have a modest impact on survival which cannot be observed outside of cohorts with vast number of patients. This discrepancy may be explained by the comparatively higher

dose and often parenteral route of broad-spectrum antibiotics delivered in clinically apparent infection, by the significant confounder of further antibiotics delivered to patients not treated with prophylaxis, and by our cohort lacking sufficient power to achieve statistical significance. Additional potential limitations include PJP prophylaxis potentially impeding opportunistic infections while preserving beneficial bacteria within the microbiome, or heterogeneity in response between the analysed groups. On the other hand, we did not demonstrate any differences in nonprophylactic antibiotic uptake between groups treated and not treated with PJP prophylaxis, which reinforces its limited value in preventing clinically-apparent infections.

Practical and ethical concerns would make it difficult to develop trials limiting reactive antibiotic delivery, which limits prospective data addressing causation in clinical studies. In our hypothesis-generating study, we performed internal/external validation and robust multivariate analyses controlling for potential confounders, providing a rationale for further prospective or mechanistic studies into whether antibiotic uptake may play a causal role in compromising survival in patients treated for GBM. We acknowledge the challenge of definitively establishing causation from associative findings in clinical data. Future research using animal models or comprehensive and dynamic (over time) characterisation of the microbiome of GBM patients during and after treatment will be helpful to determine causation.

We reproduced previous findings indicating that corticosteroid administration is associated with poor survival [18]. Although these drugs impact immunity, we did not find differences in corticosteroid uptake in groups stratified by antibiotic exposures in either cohort. Antibiotic exposures remained independently predictive of survival in multivariate models including corticosteroid uptake.

The interaction between microbiota and cancer treatment response is of intense scientific interest but, in glioma, remains unexplored. The gut microbiota can affect nongastrointestinal tumours and/or their microenvironment [19]. Also, emerging evidence suggests the presence of a microbiome associated with glioma and emerging evidence suggests that tumour-associated bacteria impact cancer outcomes [20,21]. Further research addressing this hypothesis is essential to elucidate potential causal relationships between antibiotic use and survival outcomes in GBM patients. If confirmed, GBM patients may benefit from bacteriotherapy interventions, such as faecal microbiome transplants to mitigate antibiotic-related microbiome modulation [22]. In the absence of more definitive studies, our study supports rigorous antibiotic stewardship during oncological treatment to maximise patient survival [23].

Alternative explanations beyond microbiome-mediated effects have also been considered. One possibility is that antibiotic exposure serves as a broader surrogate for treatment tolerance. Use of AC was lower in the antibiotic group within the validation cohort, although this pattern was not observed in the discovery cohort. Despite these differences, the association between antibiotic use and reduced survival was observed in both cohorts, and AC was included in multivariate models where relevant. In addition, there were no significant differences in comorbidities or PS between groups in either cohort. Alternatively, clinical infection itself may trigger an immune cascade capable of promoting tumour growth or treatment resistance [24].

We recognise other limitations. Being retrospective, this study includes patients treated according to local protocols, reflective of real-world treatment. Extent of resection and adjuvant chemotherapy were over-represented in the 'no-antibiotics' groups of the discovery and validation cohorts, respectively. Rates of AC use may reflect differences in treatment tolerances. However, these group discrepancies were different between cohorts that showed the same association between antibiotic uptake and survival. Also, these variables were included in our multivariate models. As discussed above, we recognise that our findings are hypothesis-generating rather than definitive, but they justify further research into the role of the microbiome in high-grade glioma management.

Conclusion

Our findings suggest that broad-spectrum antibiotic uptake during oncological treatment for GBM may adversely affect survival. Our results support good antibiotic stewardship to rationalise the use of broad-spectrum antibiotics in this patient group. Future research dissecting the role of the microbiome in GBM treatment response is warranted.

CRediT author contribution statement

Conceptualisation: MRF, AS, SM.
Methodology: MRF, SM.
Data collection and curation, SM, AS, JC, EC.
Formal analysis: SM, MRF.
Writing – Original Draft Preparation: SM, MRF.
Writing – Review & Editing: SM, MRF, AS, JL, OA-S, LB, KC, VM, JC, EC.
Supervision: MRF, AS.

Data statement

Data used in this study will be made available upon reasonable request by contacting directly the corresponding author.

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Declaration of competing interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clon.2026.104209.

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