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Antimicrobial prescribing in adults with acute respiratory infections following rapid six-virus antigen testing: A prospective cohort study in Italian primary care

Andrea Orsi

andrea.orsi@unige.it

Department of Health Sciences (DISSAL), University of Genoa

Alexander Domnich

IRCCS Azienda Ospedaliera Metropolitana

Antonio Vena

Department of Health Sciences (DISSAL), University of Genoa

Valentina Ricucci

IRCCS Azienda Ospedaliera Metropolitana

Matilde Ogliastro

IRCCS Azienda Ospedaliera Metropolitana

Carlo-Simone Trombetta

Department of Health Sciences (DISSAL), University of Genoa

Giulia Canepa

Department of Health Sciences (DISSAL), University of Genoa

Donatella Panatto

Department of Health Sciences (DISSAL), University of Genoa

Andrea Carraro

General Practitioner, Local Health Unit 3

Matteo Bassetti

Department of Health Sciences (DISSAL), University of Genoa

Giancarlo Icardi

Department of Health Sciences (DISSAL), University of Genoa

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Abstract

Background

The overuse of antibiotics for acute respiratory infections (ARIs) is a global challenge. While multiplex rapid antigen detection tests (RADTs) have been proposed to reduce clinical uncertainty, evidence regarding their impact on antimicrobial prescribing in outpatient settings is limited. We aimed to evaluate the association between multiplex RADT results and antimicrobial prescription rates in adult outpatients with ARIs.

Methods

This prospective cohort study was conducted in Genoa, Italy, during the 2025/2026 respiratory season. Community-dwelling adults (≥ 18 years) presenting to 27 general practitioners with an ARI were enrolled and followed for up to 30 days. At the index visit, patients underwent two nasopharyngeal swabs. The first was tested using a multiplex RADT targeting influenza A and B, respiratory syncytial virus (RSV), SARS-CoV-2, metapneumovirus, and rhinovirus. The second swab was sent to a laboratory for testing via standard-of-care real-time polymerase chain reaction (RT-PCR). The relative risk of antimicrobial prescribing between RADT-positive and RADT-negative patients was estimated using modified Poisson regression, adjusting for potential confounders.

Results

Of 682 patients tested by RADT, 18.6% tested positive for ≥ 1 virus; most cases were due to influenza A (7.8%) and RSV (5.9%). Compared to RT-PCR, the RADT showed low-to-moderate sensitivity (ranging from 0.7% for rhinovirus to 77.8% for SARS-CoV-2) and high specificity (> 99%). Sensitivity was higher for samples collected ≤ 3 days of symptom onset. Antibiotics were prescribed to 26.0% of RADT-positive and 33.9% of RADT-negative patients, with an adjusted relative risk of 0.73 (95% CI: 0.54–0.99). Antibiotic use was driven more by RADT results than RT-PCR: rates for true and false negatives (34.4% and 33.5%) were comparable, exceeding those for true and false positives (26.2% and 20.0%). All six antiviral prescriptions occurred among RADT-positive individuals.

Conclusions

A positive multiplex RADT result was associated with slightly reduced antibiotic prescribing and increased antiviral use. However, low-to-moderate sensitivity limits its utility as a universal rule-out tool. Multiplex RADTs could be best deployed as rule-in diagnostics for early presenters and high-risk patients who would benefit from prompt antiviral therapy, rather than as a general strategy to reduce overall antibiotic consumption.

Clinical trial number:

not applicable.

Introduction

Overuse of antibiotics is a global problem; inappropriate antibiotic use accounts for one-third of prescriptions and drives antimicrobial resistance [1], a leading public health crisis associated with nearly five million deaths annually [2]. Despite the known risks of antimicrobial resistance, it has been estimated that by 2030 the global antibiotic consumption will increase by 52% [3].

Acute respiratory infections (ARIs), particularly of the upper tract presenting with cough, are the top reason for primary care visits [4]. Respiratory viruses, such as SARS-CoV-2, influenza A (FluA) and B (FluB), respiratory syncytial virus (RSV), human metapneumovirus (HMPV), human rhinovirus (HRV), adenovirus, parainfluenza viruses, and seasonal coronaviruses are the most common causes of ARI in adults [5, 6]. Because the clinical presentations of viral and bacterial respiratory infections often overlap, there is a well-documented trend of overusing antibiotics and underusing antivirals [7]. US data indicate that outpatient ARIs account for 221 antibiotic prescriptions per 1000 individuals annually, with approximately 50% estimated to be clinically inappropriate [8]. In another US study [9], among ARI outpatients with laboratory-confirmed influenza, 30% were prescribed antibiotics, while only 16% received antiviral medications.

In theory, early etiologic diagnosis of ARIs could optimize antimicrobial prescribing, thereby improving patient management and outcomes [10]. While laboratory-based real-time polymerase chain reaction (RT-PCR) remains the reference standard for the detection of respiratory viruses [11], its approximately two-day turnaround time for send-out results [12] may reduce its utility in outpatient care, as it is not timely enough to inform real-time clinical decisions [13]. To address these delays, an increasing number of point-of-care test (POCT) options have become available; these can be performed via either rapid antigen detection tests (RADTs) or RT-PCR, allowing for the rapid detection of one (singleplex) or more (multiplex) respiratory pathogens [10]. While the diagnostic accuracy of many rapid multiplex RT-PCR kits is similar to that of laboratory-based assays [14], their relatively high price and limited cost-effectiveness data in real-world conditions are major barriers to widespread implementation in primary care [15]. Conversely, RADTs are cheaper, faster, and easier to perform, but their diagnostic accuracy may be low. A recent review [16] highlighted sparse evidence for the effectiveness of multiplex RADTs in primary care and identified no primary studies investigating their impact on antimicrobial prescription patterns. Their review of international guidelines also noted that France and Australia do not recommend routine multiplex RADT use [16]. To address this gap in evidence, this prospective study aimed to evaluate and compare antimicrobial prescription rates based on multiplex RADT results.

Methods

Study design, setting, and population

This prospective cohort study was conducted in Genoa (Italy) between ISO weeks 46/2025 and 17/2026. Community-dwelling adults aged ≥ 18 years seeking care for ARI were enrolled by 27 sentinel general practitioners (GPs). ARI was defined according to the current European criteria as a sudden onset of ≥ 1 respiratory symptom (cough, sore throat, shortness of breath, coryza) plus clinical judgement of an underlying infection [17]. Subjects living in long-term care facilities or those presenting with symptoms lasting more than seven days prior to the GP visit were excluded.

The study used a pragmatic recruitment strategy based on historical enrollment rates in the same area [18]. An enrollment of 20–30 patients per GP was anticipated, yielding an expected total cohort of 540–810 subjects. This sample size was previously shown to be sufficient for characterizing the local burden and diversity of respiratory pathogens [18].

Study procedures

Following screening for eligibility and obtaining written informed consent, GPs collected demographic, anamnestic, and medical history data, as well as clinical data relevant to the current ARI episode (Supplementary File 1, Table S1). Two nasopharyngeal swabs were then taken. The first swab was tested in a CE-marked six-in-one lateral flow RADT (COVID-19/Flu A + B/RSV/hRV/hMPV Ag Rapid Test by Xiamen Winbio Biotechnology; Xiamen, China) in the near-patient modality. Results were interpreted by GPs based on the appearance of the test line and the control line, according to the manufacturer's instructions. The manufacturer claimed sensitivities varying from 91.4% for HMPV to 95.7% for SARS-CoV-2, while the declared specificity was > 98% for all viruses (Supplementary File 1, Table S2). However, during an initial training, all GPs were advised of the intrinsic limitations of RADT and its suboptimal diagnostic accuracy, especially regarding the risk of false-negative results in patients with a low viral load, and that real-world performance may be lower than reported by the manufacturer. GPs received no training regarding antimicrobial stewardship, relevant clinical guidelines, or related topics.

The second swab was eluted in Universal Transport Medium (Copan Italia; Brescia, Italy) and sent to the laboratory for molecular testing. RT-PCR was performed using the Allplex Respiratory Panels 1–4 [FluA, A(H1N1)pdm09, A(H3N2) and FluB; adenovirus; human enterovirus; HMPV; parainfluenza viruses 1–4; bocavirus; seasonal coronaviruses 229E, NL63, and OC43; HRV; *Streptococcus pneumoniae*; *Bordetella pertussis*; *Bordetella parapertussis*; *Chlamydophila pneumoniae*; *Haemophilus influenzae*; *Legionella pneumophila*; *Mycoplasma pneumoniae*] and SARS-CoV-2 assays according to the manufacturer's instructions and as previously described [18]. GPs received RT-PCR results about one week later.

Fourteen (± 2) days after the initial GP visit, all patients were interviewed by a trained physician. The aim was to collect data on medications (type/brand, dosage, duration) taken, including antibiotics and antivirals; follow-up GP, emergency department, and specialist visits; diagnostic tests; complications, including hospitalizations; and workdays lost. Patients who had fully recovered (defined as a return to

their premorbid state) by day 14 concluded the study, while those not fully recovered were contacted on day 30 (± 1) to collect the same information. Analysis of clinical outcomes and economic consequences is outside the scope of this paper and will be conducted after the second season.

Study variables

The primary outcomes were prescriptions of antivirals or antibiotics during the ARI episode. The outcomes were analyzed overall and categorized by major pharmacological classes. The independent variable of interest was the RADT result (positive for ≥ 1 virus vs. pan-negative). An exploratory analysis for individual viruses was also conducted. The following variables were considered as potential confounders: age, sex, nationality, month of swabbing, swab delay (days from symptom onset to swabbing), current smoking status, presence of comorbidities, signs and symptoms recorded during the initial GP visit, and influenza and COVID-19 vaccination status.

Statistical analysis

Continuous and count variables were expressed as medians with interquartile ranges (IQRs), whereas categorical variables were expressed as proportions with exact 95% confidence intervals (CIs). Sensitivity, specificity, positive (PPV) and negative predictive (NPV) values of the RADT were computed by comparing test results to the reference standard RT-PCR. Sensitivity was also estimated by viral load (proxied by cycle threshold values, where < 30 indicates high load and ≥ 30 indicates low load) and swab delay (0–3 vs. 4–7 days). The Fisher's exact test was used to compare antimicrobial prescriptions between RADT-positive and RADT-negative individuals.

We used a modified Poisson regression approach [19] to estimate the association between antibiotic prescriptions and RADT positivity. Univariable and multivariable models were performed to calculate crude (RR) and adjusted relative risks (aRR), respectively. All models accounted for the clustering of patients within GPs by employing cluster-robust standard errors (SEs) calculated via an HC3-type covariance estimator. A purposeful selection strategy was used to build multivariable models. First, we conducted univariable analyses for all demographic and clinical variables. A preliminary multivariable model was then constructed, incorporating all covariates with $p < 0.25$. We proceeded with an iterative backward elimination process, during which variables were removed if they were non-significant and their removal resulted in an aRR change for the primary RADT exposure of < 10%. Variables were retained in the model if they maintained a $p < 0.10$ or acted as significant confounders. Finally, all two-way interaction terms were evaluated, and those found to be statistically significant were retained in the final model to account for conditional associations. Robustness of the primary findings was verified by a sensitivity analysis using a generalized estimating equation (GEE) framework with a Poisson distribution, which integrates the clustering directly into the estimation process. To account for potential informative cluster size, we specified both exchangeable and independent working correlation structures [18].

Finally, we conducted an exploratory analysis to assess virus-specific associations with antibiotic prescribing by replacing the binary RADT indicator in the final multivariable modified Poisson model with a categorical variable representing detected viruses, using pan-negative subjects as the reference group. To avoid potential bias from sparse data, samples with rare or viral co-detections resulting in subgroup frequencies < 10 were excluded from the pathogen-specific analysis. Specifically, eight RADT-positive samples (five positive for HRV, one positive for FluB, one positive for both FluA and RSV, and one positive for both FluB and RSV) were excluded.

Statistical analysis was performed using the R environment, v. 4.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of the study population

During the study period, 689 individuals were recruited. Of these, seven (1.0%) were excluded with reasons (*N* = 2 did not meet the ARI criteria; *N* = 3 were < 18 years; *N* = 2 were swabbed > 7 days after symptom onset). Nearly two-thirds (62.9%) of the enrollees were women, and the median age was 58 (IQR: 40–70) years. Approximately half (49.7%) of the outpatients had ≥ 1 comorbidity with cardiovascular (27.1%) and respiratory diseases (17.7%) being the most common. The number of samples collected peaked in December 2025 (26.5%), and the median period between sample collection and symptom onset was three days. Vaccine uptake for the 2025/2026 season influenza and COVID-19 was 41.5% and 2.5%, respectively. At the index GP visit, malaise (85.8%), fever/feverishness (65.5%), and myalgia (65.2%) were the most common systemic symptoms, while cough (92.5%) and coryza (86.8%) were the most frequent respiratory complaints (Table 1).

Table 1
Characteristics of the study population at enrollment (*N* = 682); Genoa (Italy), 2025/2026 respiratory season

Variable	Level	*N* (%)	95% CI
Sex	Female	429 (62.9)	59.2–66.5
	Male	253 (37.1)	33.5–40.8
Age, years	Median [IQR]	58 [40–70]	–
	18–59	354 (51.9)	48.1–55.7
	≥ 60	328 (48.1)	44.3–51.9
Nationality	Italian	620 (90.9)	88.5–93.0
	Foreign	62 (9.1)	7.0–11.5
Month of swab	November 2025	98 (14.4)	11.8–17.2
	December 2025	181 (26.5)	23.3–30.0
	January 2026	119 (17.4)	14.7–20.5
	February 2026	124 (18.2)	15.4–21.3
	March 2026	129 (18.9)	16.0–22.1
	April 2026	31 (4.5)	3.1–6.4
Swab delay, days	Median [IQR]	3 [2–4]	–
Current smoking	No	548 (80.4)	77.2–83.3
	Yes	134 (19.6)	16.7–22.8
Comorbidities	≥ 1	339 (49.7)	45.9–53.5
	Cardiovascular	185 (27.1)	23.8–30.6

Variable	Level	*N* (%)	95% CI
	Respiratory	121 (17.7)	14.9–20.8
	Diabetes	47 (6.9)	5.1–9.1
	Hepatic	15 (2.2)	1.2–3.6
	Renal	23 (3.4)	2.1–5.0
	Cancer/immunosuppression	73 (10.7)	8.5–13.3
	Anemia	24 (3.5)	2.3–5.2
	Obesity	50 (7.3)	5.5–9.6
	Dementia	12 (1.8)	0.9–3.1
Signs and symptoms at presentation	Fever/feverishness	447 (65.5)	61.8–69.1
	Shivering	420 (61.6)	57.8–65.3
	Headache	423 (62.0)	58.3–65.7
	Myalgia	445 (65.2)	61.5–68.8
	Arthralgia	418 (61.3)	57.5–65.0
	Malaise	585 (85.8)	82.9–88.3
	Decreased appetite	286 (41.9)	38.2–45.7
	Nausea	125 (18.3)	15.5–21.4
	Diarrhea	75 (11.0)	8.7–13.6
	Cough	631 (92.5)	90.3–94.4
	Sputum production	397 (58.2)	54.4–61.9
	Dyspnea	94 (13.8)	11.3–16.6
	Rhonchi	110 (16.1)	13.4–19.1
	Wheezing	64 (9.4)	7.3–11.8

Variable	Level	*N* (%)	95% CI
	Low/decreased SaO2	13 (1.9)	1.0–3.2
	Sore throat	427 (62.6)	58.9–66.3
	Coryza	592 (86.8)	84.0–89.3
	Altered smell	160 (23.5)	20.3–26.8
	Altered taste	130 (19.1)	16.1–22.2
2025/2026 season influenza vaccination	No	399 (58.5)	54.7–62.2
	Yes	283 (41.5)	37.8–45.3
2025/2026 season COVID-19 vaccination	No	665 (97.5)	96.0–98.5
	Yes	17 (2.5)	1.5–4.0
Total COVID-19 vaccine doses	Median [IQR]	3 [3–3]	–

IQR, interquartile range.

Detection rates of respiratory pathogens and RADT diagnostic accuracy

Using RADT, ≥ 1 virus was detected in 127 of 682 samples (18.6%; 95% CI: 15.8–21.8%). Of these, 125 were single detections, and two samples displayed double positivity (FluA/RSV and FluB/RSV). Detections of FluA (7.8%) and RSV (5.9%) were the most common, whereas HMPV (2.2%), SARS-CoV-2 (2.1%), HRV (0.7%), and FluB (0.3%) were less frequent. Most SARS-CoV-2 detections occurred in November and December 2025, while FluA was detected between November 2025 through March 2026, peaking in December 2025. RSV and HMPV peaked later during the season (Fig. 1).

When tested by RT-PCR, ≥ 1 pathogen was detected in 518 samples (76.0%; 95%: 72.6–79.1%). Considering only the six shared viruses, detection rates were higher by RT-PCR than by RADT for FluA (18.5%), RSV (10.3%), HMPV (6.0%), and HRV (22.0%), while the frequencies of SARS-CoV-2 (2.6%) and FluB (0.1%) were comparable (Fig. 1).

Compared to the RT-PCR reference standard, RADT showed moderate-to-low sensitivity for SARS-CoV-2 (77.8%), RSV (50.0%), FluA (40.5%), and HMPV (36.6%). Conversely, RADT showed negligible sensitivity (0.7%) for HRV. As there was only one true-positive FluB sample, which RADT identified correctly, the

sensitivity (100%) for FluB was not considered meaningful. RADT specificity was > 99% for all targets. PPVs and NPVs ranged 25.0–100% and 78.0–100%, respectively (Supplementary File 1, Table S2). As expected, RADT sensitivity was greater for samples with high viral load and those collected within the first three days after symptom onset (Supplementary File 1, Table S3).

Legend

The stacked bar charts (left panels) display the absolute number of monthly pathogen detections by rapid antigen detection test (upper panels) and real-time polymerase chain reaction (lower panels). The pie charts (right panels) display the cumulative distribution of these detections across the entire study period. Numbers may not sum to total patients due to the presence of co-detections.

Prescription of antimicrobials by rapid antigen detection test result

Follow-up data were available for 675 (99.0%) subjects; seven individuals could not be reached. Six antiviral prescriptions were recorded overall: five for oseltamivir and one for nirmatrelvir/ritonavir. All prescriptions were restricted to RADT-positive patients [4.7% (6/127); 95% CI: 1.8–10.0%], with no prescriptions [0% (0/548); 95% CI: 0–0.7%] among RADT-negative individuals ($p < 0.001$). The specific prescription rates among RADT-positive cases were 9.4% (5/53) for oseltamivir among FluA cases and 7.1% (1/14) for nirmatrelvir/ritonavir among SARS-CoV-2 cases. Due to the low number of antiviral prescriptions, no further analyses were conducted.

Regarding antibiotics, ≥ 1 prescription occurred in one third of patients (32.4%; 219/675) corresponding to 240 total prescriptions. Indeed, 198 (90.4%) and 21 (9.6%) patients were prescribed one and two antibiotic types, respectively. Of the total prescriptions, amoxicillin/clavulanic acid was the most common type (43.3%; 104/240), followed by macrolides (36.3%; 87/240) and cephalosporins (13.8%; 33/240) (Supplementary File 1, Table S4).

Overall, antibiotic prescriptions were tendentially more frequent ($p = 0.093$) among subjects who tested negative (33.9%; 186/548) by RADT compared to those who tested positive for ≥ 1 virus (26.0%; 33/127); the crude the RR was 0.77 (95% CI: 0.55–1.07; $p = 0.11$). In the final multivariable model, after adjusting for potential confounders, the RR was 0.73 (95% CI: 0.54–0.99; $p = 0.041$). Other significant independent predictors of antibiotic prescription included the presence of fever/feverishness (aRR 1.44; 95% CI: 1.14–1.82), decreased appetite (aRR 1.25; 95% CI: 1.02–1.54), cough (aRR 2.09; 95% CI: 1.08–4.07), and rhonchi (aRR 2.52; 95% CI: 2.06–3.09). Furthermore, a significant ($p = 0.031$) interaction term between diabetes and swab delay was identified (aRR 1.42; 95% CI: 1.03–1.95), indicating that for patients with diabetes, each additional day of delay in testing was associated with a statistically significant increase in the risk of receiving an antibiotic prescription (Fig. 2; Supplementary File 1, Table S5). These results were robust in the sensitivity analysis when GEE models were applied and the effect sizes were almost identical (Fig. 2; Supplementary File 1, Table S6).

Figure 2 Relative risks for antibiotic prescriptions among patients positive by RADT versus pan-negative subjects

When reconciling results of RADT and RT-PCR for six overlapping viruses, it emerged that antibiotic prescription rates were more similar by the RADT result: the rates for RADT true negatives (34.4%; 100/291) and false negatives (33.5%; 86/257) were comparable and higher than the rates for RADT true positives (26.2%; 32/122) and false positives (20.0%; 1/5).

In an exploratory analysis on the association between antibiotic prescriptions and specific viral pathogens identified via RADT, SARS-CoV-2 (aRR 0.24, 95% CI: 0.02–2.43), FluA (aRR 0.74, 95% CI: 0.52–1.05), RSV (aRR 0.62, 95% CI: 0.35–1.12), and HMPV (aRR 0.88, 95% CI: 0.26–2.95) showed similar trends toward reduced antibiotic prescribing compared to pan-negative patients. However, these results were associated with large uncertainty due to limited virus-specific positivity rates (Supplementary File 1, Table S7).

Discussion

This study evaluated antimicrobial prescribing patterns among adult outpatients seeking primary care for ARI, comparing prescription rates between patients who test positive on a multiplex RADT targeting six common respiratory viruses and those who test negative. Our findings reveal that despite the moderate-to-low sensitivity of the RADT, testing positive was associated with a 27% reduction in antibiotic prescriptions. Furthermore, while RADT positivity increased antiviral prescribing, particularly for influenza, the limited number of positive cases precluded definitive statistical interpretation.

The outpatient setting accounts for the majority of antibiotic prescriptions; however, the utility of POCTs, RADTs in particular, in primary care remains underexplored. Outpatients generally present with less severe respiratory illness compared to hospitalized or emergency department (ED) cohorts, where most existing POCT research is concentrated [20–24]. The available meta-analyses of randomized controlled trials (RCTs) underscore the inconsistency of the impact of POCT on antibiotic prescribing, with most studies failing to demonstrate a significant effect [22–24]. For instance, Li et al. [22] found that molecular POCT had little to no effect on antibiotic use compared with usual care in patients of all ages (RR 0.95; 95% CI: 0.90–1.00); this effect remained null when the analysis was restricted to adults (RR 1.00; 95% CI: 0.98–1.02). Conversely, molecular POCT was associated with an increase in appropriate antibiotic prescribing (RR 2.07; 95% CI: 1.55–2.77), defined as the use of agents with in vitro activity against the detected pathogen and of the narrowest possible spectrum [22]. An earlier meta-analysis focusing on influenza POCTs (mostly pediatric ED patients) reported no significant effect on antibiotic prescribing across seven RCTs (RR 0.97; 95% CI: 0.82–1.15) [23]. Notably, results differed significantly in non-randomized studies, where a pooled RR of 0.64 (95% CI: 0.48–0.86) was observed across five studies, four of which reported significant reductions in antibiotic use. The authors attributed these conflicting findings to variations in baseline prescribing rates, influenza attack rates, and a higher risk of bias in non-randomized designs [23]. These aggregate null findings, however, may obscure complex

clinician behavioral responses that only emerge upon closer inspection of test results. This has been recently illustrated in an English RCT [25], where participants were randomized to either an intervention group receiving a 23-plex molecular POCT or a control group receiving usual care. Antibiotics were prescribed to 45% of patients in both groups [odds ratio (OR) 1.00; 95% CI: 0.71–1.41]. However, a prespecified subgroup analysis revealed that among patients with a detected virus, antibiotic prescribing was significantly reduced in the intervention group compared to the control group (22% vs. 46%; OR 0.35; 95% CI: 0.20–0.63). Conversely, in instances where no virus was detected, antibiotic prescribing increased (61% vs. 45%; OR 1.89; 95% CI: 1.20–2.96). The authors concluded that the decrease in antibiotic use observed in viral-positive patients was entirely offset by compensatory prescribing when no virus was detected, suggesting that a positive viral result increases physician confidence to withhold antibiotics [25]. A similar pattern is evident in a pediatric meta-analysis [26], where the impact of diagnostic testing on prescribing behavior depends entirely on the chosen comparator. While comparisons of rapid influenza testing versus clinical diagnosis showed no significant effect on antibiotic prescribing (OR 0.64; 95% CI: 0.36–1.15), the effect became statistically significant when comparing patients with positive versus negative rapid test results (OR 0.35; 95% CI: 0.21–0.57) or when comparing rapid test-positive patients against those confirmed positive via standard-of-care testing (OR 0.43; 95% CI: 0.25–0.74).

In our study, all antiviral prescriptions occurred among subjects who tested positive for FluA or SARS-CoV-2, whereas no such prescriptions were made among subjects who tested pan-negative. While these data suggest that RADT could facilitate targeted antiviral initiation, the number of prescriptions was low, precluding adjusted analyses. Contrary to the complexities surrounding antibiotic use, the available literature on the impact of POCT on antiviral prescribing is more homogeneous, generally suggesting an increase in prescribing. However, as with antibiotic endpoints, the findings are highly contingent upon the comparator used. For instance, Lee et al. [23] reported a significant increase in antiviral prescribing in RCTs (RR 2.65; 95% CI: 1.95–3.60) and a similarly high, albeit more variable, effect in non-randomized studies (RR 11.36; 95% CI: 0.82–157.12). Similarly, Clark et al. [27] found that influenza-positive patients were significantly more likely to receive neuraminidase inhibitors when tested with rapid multiplex PCR compared to routine testing (RR 1.25; 95% CI: 1.06–1.48). In contrast, when the comparison is shifted to a POCT versus usual care design that disregards individual test results, the effect is often null. Indeed, the meta-analysis by Li et al. [22] showed no effect on antiviral prescribing (RR 1.01; 95% CI: 0.82–1.25).

As noted earlier, the available international recommendations generally do not support the routine use of multiplex RADTs due to concerns regarding insufficient diagnostic performance, though the supporting evidence remains limited [16]. Similarly, Italian Ministry of Health guidelines (last updated in 2010) [28] do not recommend the routine use of rapid influenza testing in outpatients with influenza-like illness (ILI), citing concerns of low PPV and the inability of a negative result to reliably exclude infection, which may lead to clinical uncertainty. However, these guidelines acknowledge the high variability of PPV based on influenza attack rates and that prior evidence was largely derived from hospital settings, limiting its generalizability to primary care [28]. In line with these recommendations, in this study the observed RADT sensitivity ranged from almost null for HRV, low for FluA, RSV, and HMPV, and moderate for SARS-

CoV-2. Notably, the observed sensitivity was lower than that reported by the manufacturer. This divergence is common [29], as manufacturer-sponsored validation studies are often optimized via purposive sampling of well-characterized specimens, which may introduce selection bias and inflate diagnostic accuracy parameters. As expected, sensitivity was higher for samples collected within the first three days following symptom onset, while the false-negative rate was particularly high for samples swabbed 4–7 days after symptom onset. This decline in sensitivity paralleled the observed trends in prescribing: antibiotic prescriptions trended higher as time since symptom onset increased, whereas all antiviral prescriptions were confined to the first three days. Considering these findings alongside the high specificity observed across all targets, we believe that multiplex RADTs should not be used as universal tools for adult outpatients with ARI. Instead, they can function most effectively as rule-in diagnostic tools for early presenters, particularly those at high risk who may benefit from prompt antiviral therapy, and when used during periods of high viral circulation. Before their implementation, multiplex RADTs should be independently evaluated for their real-world performance.

The alignment of our findings with broader clinical practice is supported by comparing our antibiotic prescription rates for RADT-negative subjects (33.9%) with historical data from the Italian outpatient landscape. In the largest representative Italian study on the natural history of clinically diagnosed influenza, antibiotics were prescribed to 35.6% of adults [30], while a subsequent cross-sectional telephone survey of patients reported a rate of 37.6% [31]. These comparable proportions further support the idea that the RADT could function better as a rule-in rather than a rule-out diagnostic tool.

Diagnostic uncertainty remains a primary driver of antibiotic overprescription for outpatients presenting with ARI [32]. Even when diagnostic tests confirm a viral etiology, clinicians may still prescribe antibiotics due to concerns regarding potential bacterial co-infection or secondary superinfection. For instance, Steger et al. [33] found that 23.6% of adults with RT-PCR-confirmed influenza received antibiotic therapy. This figure aligns closely with our findings, in which 26.0% of RADT-positive patients were prescribed antibiotics. Notably, we observed that, independently of RADT results, the presence of rhonchi was the strongest predictor of antibiotic prescription, suggesting that GPs may interpret these findings as evidence of a bacterial process in the lower respiratory tract. It is well-recognized that antibiotics are frequently overused for ARIs in primary care. However, estimates of the inappropriate use vary widely, ranging from 13% to 90% across individual studies [34]. This heterogeneity is driven, in part, by the specific definition of "appropriateness" used across studies. Theoretically, the appropriateness of antibiotic prescribing can be assessed by comparing patient-level data against clinical guidelines or by benchmarking observed prescribing rates against "ideal" target rates [35]. According to Italian guidelines [28], antibiotics should not be used for uncomplicated ILI in primary care. In this context, the above-described Italian study found that 35.1% of patients with clinically diagnosed influenza developed ≥ 1 complication, a figure that closely mirrored the 35.6% antibiotic prescription rate, leading the authors to conclude that the prescribing was likely appropriate [30]. Conversely, when applying the benchmark method, acceptable antibiotic prescribing ranges in Europe have been estimated at 0–30% for patients aged 18–75 years with acute bronchitis/bronchiolitis, and 0–20% for ARI patients aged ≥ 1 year [36]. Importantly, we did not attempt to categorize prescriptions as appropriate or inappropriate, as such

an assessment requires ancillary clinical and laboratory data that were not available and were beyond the scope of our primary objective.

Interpretation of our findings must consider several limitations. First, several point estimates were associated with wide CIs due to limited statistical power. The low frequency of antiviral prescriptions and virus-specific positive cases limited our ability to perform granular subgroup analyses or adjust for potential confounders. Second, the results of this study may not be generalizable to seasons characterized by lower virus circulation or varying activity levels of individual viruses. For example, the study period was characterized by a high circulation of FluA, while other viruses were less common. Lower viral circulation, under the same conditions and diagnostic performance of the RADT, would decrease PPV and NPV. Third, our findings relate specifically to the chosen multiplex RADT, while the diagnostic accuracy of commercially available RADTs varies widely [37]. While our performance estimates are broadly consistent with meta-analytical accuracy estimates of singleplex RADTs in adults for SARS-CoV-2 (sensitivity 72.7%, specificity 98.7%) [37], FluA (sensitivity 42.6%, specificity 99.5%) [38], and RSV (sensitivity 29%, specificity 99%) [39], rapid molecular POCTs may have a comparable sensitivity to standard laboratory-based RT-PCR [14], potentially reducing the false-negative rate and improving antimicrobial stewardship. However, the high cost of molecular platforms currently limits their widespread adoption in primary care. Fourth, while we accounted for a comprehensive list of pre-exposure variables and employed purposeful selection of predictors, the possibility of residual confounding, stemming from unmeasured patient- or GP-level factors, remains a limitation inherent to observational research. Finally, as discussed, we considered all antibiotic prescriptions regardless of their appropriateness. For this reason, it is likely that RADT use facilitated more appropriate prescribing, which may be masked when focusing only on total prescription rates.

Conclusion

The six-plex RADT used in this study showed low-to-moderate sensitivity, which varied by viral target, viral load, and time from symptom onset. On the one hand, a positive test result was associated with a significant reduction in antibiotic prescribing and a modest increase in antiviral use. On the other hand, the high rate of false-negative results limits the clinical utility of the test. Supported by prior randomized evidence [25], we believe that a negative RADT result fails to provide sufficient reassurance to modify established empirical prescribing habits when no virus is detected, as it may paradoxically trigger compensatory antibiotic prescribing to cover for potentially overlooked bacterial infections. As such, RADTs have limited utility when applied as a universal diagnostic strategy for all patients seeking primary care for ARI, primarily due to their suboptimal sensitivity in real-world settings. Consequently, multiplex RADTs could be deployed as rule-in diagnostic tools specifically for early presenters and high-risk patients who may derive direct clinical benefit from the prompt initiation of targeted antiviral therapy. Ultimately, the value of these tests is contingent upon the clinical context rather than their inherent diagnostic accuracy.

Abbreviations

ARI	Acute respiratory infection
aRR	Adjusted relative risk
CI	Confidence interval
ED	Emergency department
FluA	Influenza A
FluB	Influenza B
GEE	Generalized estimating equation
GP	General practitioner
HMPV	Human metapneumovirus
HRV	Human rhinovirus
ILI	Influenza-like illness
IQR	Interquartile range
NPV	Negative predictive value
OR	Odds ratio
POCT	Point-of-care test
PPV	Positive predictive value
RADT	Rapid antigen detection test
RCT	Randomized controlled trial
RR	Relative risk
RSV	Respiratory syncytial virus
RT-PCR	Real-time polymerase chain reaction
SE	Standard error

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki (2024 revision). The study was approved by the Liguria Region Ethics Committee (protocol 397/2023, DB id 13354 of 18 September 2023 and subsequent amendment of 16 October 2025). All patients provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to CIRI-IT internal policy and local ethical restrictions but are available from the corresponding author on reasonable request.

Competing interests

A.O. provided consultancies and/or received speaker fees from CSL Seqirus, Moderna, Novavax, and SD Biosensor. A.D. provided consultancies and/or received speaker fees from CSL Seqirus, GSK, Sanofi, and SD Biosensor. A.V. provided consultancies and/or received speaker fees from Pfizer, Shionogi, Tillotts Pharma, Menarini, Gilead, Mundipharma, Advanz Pahrma, and MSD. D.P. provided consultancies for Pfizer and CSL Seqirus and received grants for conducting observational studies from Sanofi, Pfizer, GSK, and Viatris. M.B. received funding for scientific advisory boards, travel, and speaker honoraria from Cidara, Gilead, Menarini, MSD, Mundipharma, Pfizer, and Shionogi. G.I. provided consultancies and/or received grants for conducting experimental and/or observational studies for GSK, Sanofi, MSD, CSL Seqirus, and Pfizer. V.R., M.O., C.-S.T., G.C., and A.C. declare no conflicts of interest.

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Authors' contributions

A.O, A.D., A.C. and G.I. conceived and designed the study. V.R. was responsible for laboratory analyses. C.-S.T. and G.C. performed follow-up calls of the patients enrolled. M.O. and D.P. were responsible the data quality. A.O., A.D. and A.V. interpreted the results. A.O. and A.D. performed data analyses and drafted the manuscript. A.V., V.R., M.O., D.P., A.C., M.B. and G.I. critically revised the manuscript. G.I. supervised the project.

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Figures

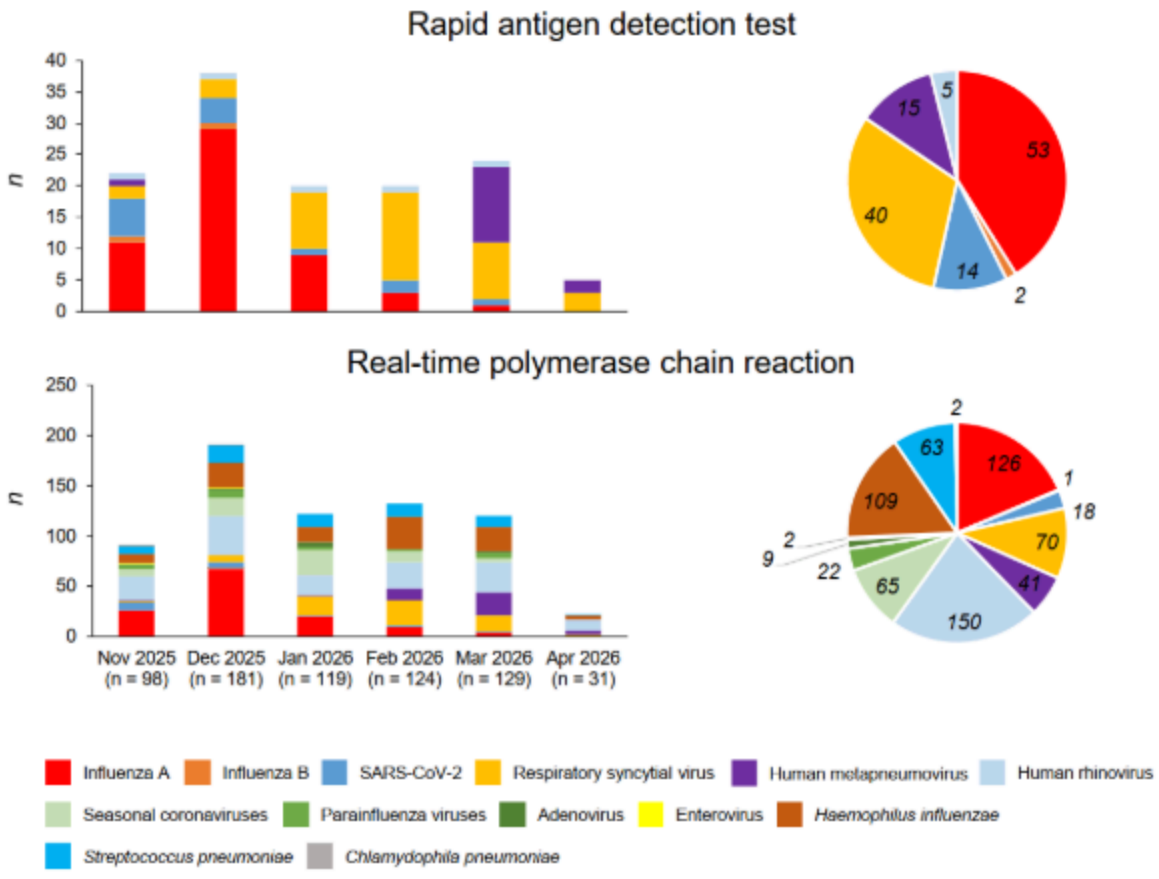

Figure 1

Monthly and cumulative distributions of respiratory pathogens, by diagnostic assay; Genoa (Italy), 2025/2026 respiratory season

The stacked bar charts (left panels) display the absolute number of monthly pathogen detections by rapid antigen detection test (upper panels) and real-time polymerase chain reaction (lower panels). The pie charts (right panels) display the cumulative distribution of these detections across the entire study period. Numbers may not sum to total patients due to the presence of co-detections.

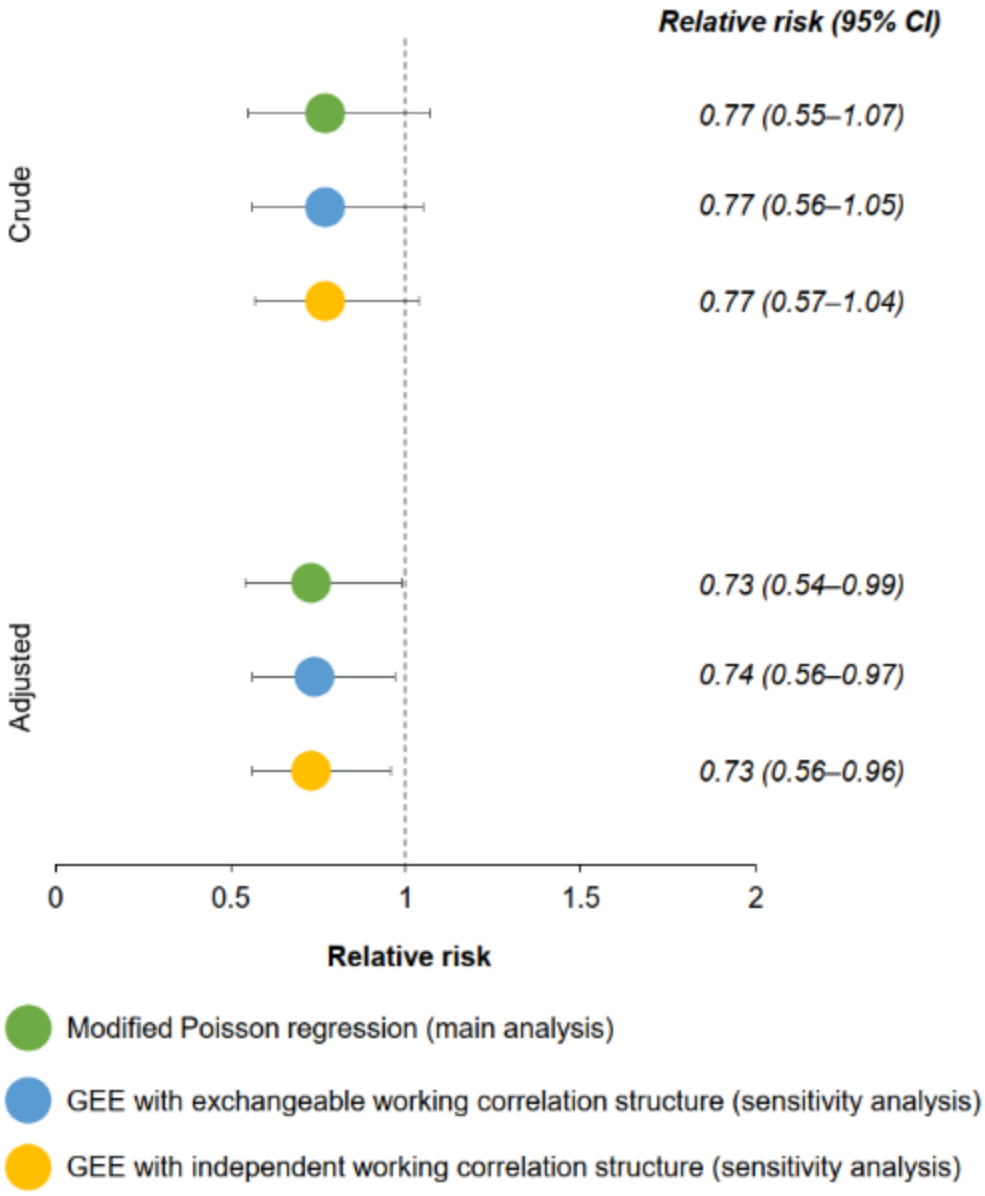

Figure 2

Relative risks for antibiotic prescriptions among patients positive by RADT versus pan-negative subjects

Abbreviations: CI, confidence interval; GEE, generalized estimating equation; RADT, rapid antigen detection test.

Crude estimates represent results from univariable models. Adjusted estimates were derived from multivariable models including the following predictors: RADT positivity for ≥ 1 virus, fever/feverishness,

decreased appetite, cough, rhonchi, altered taste, and diabetes by swab delay interaction term. The main analysis used modified Poisson regression with cluster-robust standard errors, while the sensitivity analysis employed generalized estimating equations with exchangeable and independent working correlation structures.

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