Journal of Hospital Infection 162 (2025) 26–35

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Postoperative risk of infection with klebsiella in adults – a retrospective case–control study

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ARTICLE INFO

Article history:
Received 15 November 2024
Accepted 21 April 2025
Available online 27 May 2025

Keywords:
Klebsiella
Postoperative
Infection
Case–control
Epidemiology
Incidence

SUMMARY

Background: Hospital-acquired infections with *Klebsiella* spp. and emerging multi-drug-resistant strains are a persistent concern. Identifying high-risk groups is crucial for the evaluation of preventive interventions such as vaccines. We determined the incidence and developed prediction models for postsurgical klebsiella infections in adult elective surgery patients.

Methods: This multi-centre retrospective case–control study, in seven European hospitals, included patients ≥50 years old who underwent elective surgery between 2012 and 2021. Using multi-variable logistic regression, we modelled the risk of postsurgical klebsiella infection and investigated trial enrichment scenarios.

Results: Of 139,778 eligible surgeries identified, 1781 were included: 840 patients with postsurgical klebsiella infection and 941 without. The incidence of postsurgical klebsiella infection was 1.38% (95% confidence interval 1.24–1.54%). Pre-surgical klebsiella colonization, gastrointestinal surgery, abdominal surgery, trauma surgery and chronic cardiovascular disease were independent predictors of postoperative klebsiella infection.

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https://doi.org/10.1016/j.jhin.2025.04.036

Minimally invasive surgery and peri-operative antibiotic prophylaxis predicted a lower risk. Trial enrichment simulation indicated a 72% reduction in required participants when enrolling patients with a predicted risk above 2%.

Conclusions: A multi-variable model incorporating klebsiella colonization status and clinical factors can accurately predict klebsiella infections in elective surgery patients. This model can select high-risk patients, enhancing the efficiency of phase-III trials of preventive interventions, including vaccination.

Introduction

Klebsiella spp. are prevalent pathogens in hospital-acquired infections, most commonly pneumonia and urinary tract infections, followed by bloodstream infections [1,2]. There is ubiquitous exposure to *Klebsiella* spp. in the hospital setting, facilitated through interactions with hospital staff, medical devices, and surgical procedures. Patients colonized with *Klebsiella* spp. upon admission, exhibit higher klebsiella infection rates compared with non-colonized patients [3–5]. Despite this, screening for klebsiella colonization upon hospital admission is uncommon.

In recent years, the prevalence of infections with multidrug-resistant (MDR) *Klebsiella* spp. has increased drastically [6]. Particularly concerning is the rise in infections involving carbapenemase production [7,8]. This enzyme renders carbapenem antibiotics ineffective – one of the last lines of therapy against many bacterial infections. The transfer of resistance genes among bacteria through plasmids has led to *Klebsiella pneumoniae* (KP) becoming the primary reservoir for transmissible carbapenemase genes [9]. In the European Union, a notable proportion of KP infection isolates exhibit resistance to carbapenems, with certain countries reporting alarmingly high prevalence rates [10]. The World Health Organization (WHO) has classified carbapenem-resistant Enterobacterales and extended-spectrum cephalosporin-resistant Enterobacterales (including *Klebsiella* spp.) as pathogens of critical importance for antibiotic research and development [11]. This prioritization reflects the inadequate antibiotic pipeline, the substantial healthcare burden, and the challenges in effectively preventing these infections in healthcare settings [11]. Therefore, prevention of KP infections is critical in reducing the impact of carbapenem resistance.

Current preventive interventions include pre-operative colonization screening and perioperative antibiotic prophylaxis (PAP). Recent clinical practice guidelines recommend colonization screening and targeted PAP in patients undergoing colorectal surgery and solid organ transplantation [12]. Yet, significant knowledge gaps remain regarding the effectiveness and target population of these strategies. Given the increasing prevalence of antibiotic resistance and the limitations of PAP, alternative preventive measures, such as vaccination, are gaining interest. Currently, no vaccine against *Klebsiella* spp. is available on the market, and the tetravalent bioconjugate Kleb4V is the only candidate vaccine recently evaluated in a phase-2 clinical trial [13].

To facilitate the development and evaluation of preventive interventions against *Klebsiella* spp. infections, such as vaccines, improved epidemiological insights within potential target groups are imperative. Adult elective surgery patients constitute an ideal target group to study the efficacy of targeted preventive interventions, owing to their pre-planned surgical schedules (allowing for pre-operative microbiological screening), extended postoperative follow-up periods, and a relatively high incidence of klebsiella infections. Therefore, the current study aims to estimate the cumulative incidence of klebsiella infections and identify associated risk factors in this population to provide insights for designing clinical trials evaluating the efficacy of targeted preventive interventions against klebsiella infections.

Methods

Study design

This multi-centre retrospective case–control study encompassed elective surgeries performed between 2016 and 2021 across seven hospitals: five in Italy, one in Greece, and one in Serbia. Notably, the Serbian site contributed data from elective surgeries conducted between 2012 and 2016 as part of a prospective study.

Inclusion criteria

Sites were eligible if they conducted routine pre-operative screening for *Klebsiella* spp. colonization for at least one type of surgery, could extract individual surgery records and link them to clinical cultures and maintained active clinical follow-up on postsurgical klebsiella infections. Patients were eligible if they underwent elective surgery at a participating site between 2016 and 2021, were aged 50 years or older at the time of surgery and underwent a type of surgery involving routine pre-operative screening for klebsiella colonization. We focused on patients aged $>$50 years because this population is at increased risk for postoperative infections and associated complications. Advanced age is a well-established risk factor for nosocomial infections due to immunosenescence, higher rates of comorbidities, and prolonged hospital stays [14]. Transplant patients were eligible, but their data will be reported separately because of an unusually high rate of positive post-surgical klebsiella cultures due to routine post-surgical screening, leading to potential bias in the study results. Additionally, the clinical criteria for diagnosing urinary tract infections (UTIs) were not well-suited for kidney transplant patients, presumably resulting in misclassification of infections. We also considered that the transplant population would likely not be part of the target group for a pivotal klebsiella vaccine trial. There were no exclusion criteria.

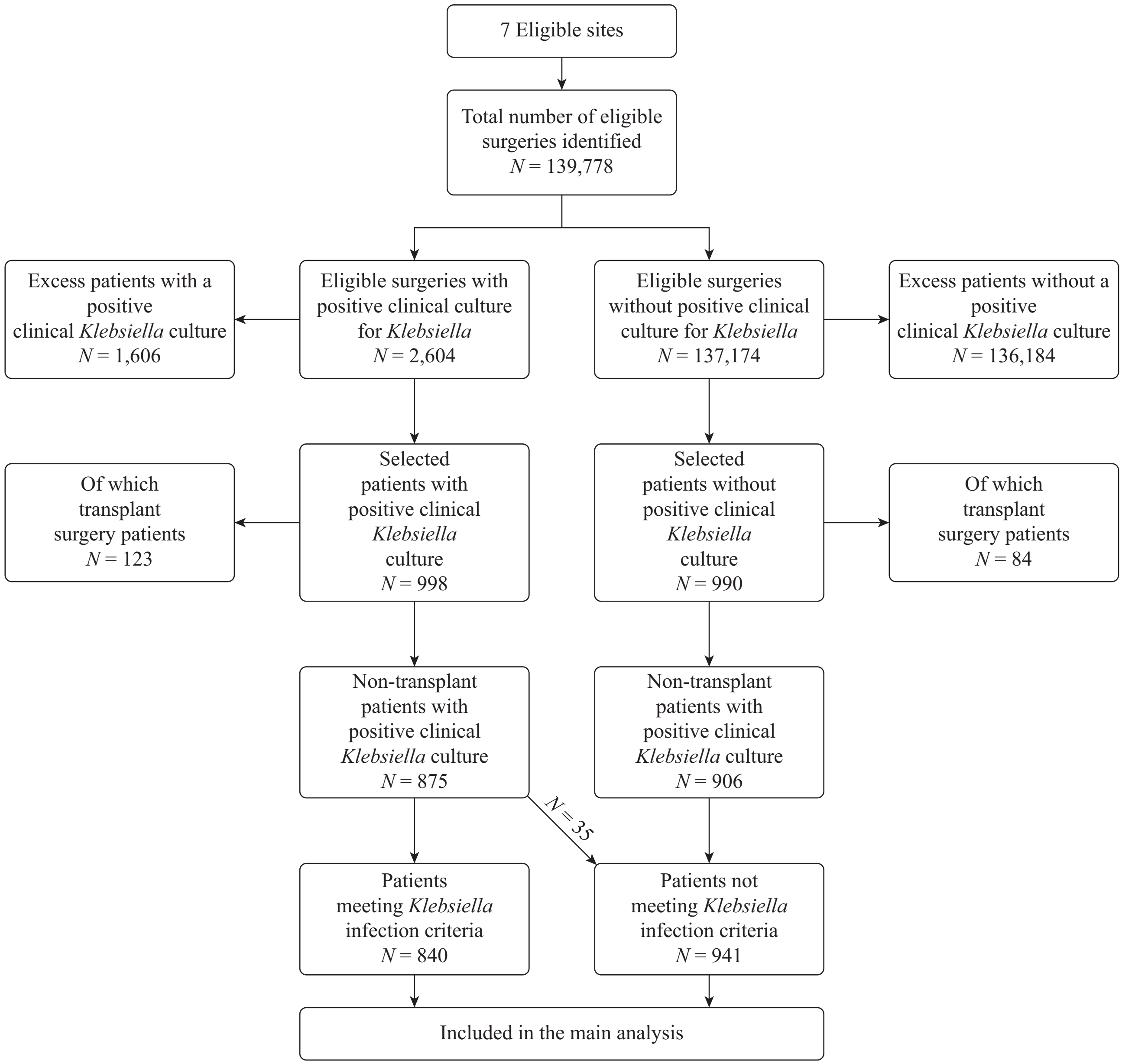

Figure 1. Flowchart of inclusion.

Patient selection and data collection

Each participating centre constructed a list of surgical procedures for which routine pre-operative screening for Klebsiella was performed. Subsequently, patients operated upon within the study period were screened on eligibility based on type of surgery and age. Eligible patients were linked to microbiology records to identify patients with identification of *Klebsiella* spp. in clinical samples by culture or polymerase chain reaction within one to 180 days after surgery. Clinical samples did not include screening. All eligible patients with a positive clinical culture were included until 1000 patients with a positive post-surgical klebsiella culture were selected for inclusion. An equal number of surgeries was randomly selected from the pool of patients without a postsurgical clinical culture positive for *Klebsiella* spp. (including patients with negative cultures, absence of cultures and cultures yielding only pathogens other than klebsiella). Baseline data included demographics, comorbidities, colonization screening results, year and type of surgery, and perioperative antibiotic prophylaxis details. Additional data for patients with a clinical culture positive for *Klebsiella* spp. included sample type, microbiological procedures, klebsiella species, resistance profile, and other identified pathogens. Infection criteria were retrospectively assessed by local researchers, using the modified criteria from the Centers for Disease Control and Prevention (CDC) National Healthcare Safety Network (NHSN) for healthcare-associated infections [15]. For patients meeting the infection criteria, we collected infection type, post-operative day of infection, and the need for hospital readmission. eCRF records were monitored remotely for completeness and consistency. Site-level data included the

Table I
Baseline characteristics of the weighted study population

	Infected[a]	Not infected[b]	Total population
Number of patients	840	941	1781
Size of the underlying population[c]	840	59,982	60,822
Age in years, mean (SD)	69.2 (9.5)	68.1 (9.2)	68.1 (9.2)
Female sex	332 (39.5%)	26711 (44.5%)	27043 (44.5%)
Medical history			
Cardiovascular disease	427 (51.1%)	30,648 (51.4%)	31,075 (51.4%)
Chronic pulmonary disease	90 (10.8%)	4531 (7.6%)	4621 (7.6%)
Diabetes mellitus	212 (25.4%)	10,615 (17.8%)	10,827 (17.9%)
Moderate to severe chronic renal failure	74 (8.9%)	2285 (3.8%)	2359 (3.9%)
Solid organ malignancy	548 (65.6%)	24,429 (40.9%)	24,977 (41.3%)
Urological diseases including prostatitis	92 (11.0%)	3414 (5.7%)	3506 (5.8%)
Indwelling urinary catheter	30 (3.6%)	1050 (1.8%)	1080 (1.8%)
Immune suppression	129 (15.4%)	4175 (7.0%)	4304 (7.1%)
Harmful use of alcohol or alcohol dependence	12 (1.4%)	124 (0.2%)	136 (0.2%)
Antibiotic use within 30 days before surgery[d]	82 (9.8%)	920 (1.5%)	1002 (1.6%)
Surgical procedure			
Cardiothoracic surgery	82 (9.8%)	13,528 (22.6%)	13,610 (22.4%)
Gastrointestinal surgery	395 (47.0%)	8974 (15.0%)	9369 (15.4%)
Other abdominal surgery	169 (20.1%)	7126 (11.9%)	7295 (12.0%)
Urological surgery	59 (7.0%)	4218 (7.0%)	4277 (7.0%)
Orthopaedic surgery	83 (9.9%)	8966 (14.9%)	9049 (14.9%)
Neurosurgery	25 (3.0%)	10,919 (18.2%)	10,944 (18.0%)
Head and neck surgery	13 (1.5%)	5245 (8.7%)	5258 (8.6%)
Obstetric and gynaecological surgery	5 (0.6%)	1007 (1.7%)	1012 (1.7%)
Other surgery	9 (1.1%)	0 (0.0%)	9 (0.0%)
Minimally invasive surgery	168 (20.0%)	23,050 (38.4%)	23,218 (38.2%)
Colonization screening			
Klebsiella colonization screening performed[e]	786 (93.6%)	56,608 (94.4%)	57,394 (94.4%)
Any *Klebsiella* spp. identified[f]	87 (11.1%)	79 (0.1%)	166 (0.3%)
Resistant to 2nd/3rd generation cephalosporins	80/81 (99%)	77/77 (100%)	157/158 (99%)
Resistant to 4th generation cephalosporins	68/72 (94%)	66/66 (100%)	134/138 (97%)
Resistant to carbapenems	68/82 (83%)	54/77 (70%)	122/159 (77%)
Resistant to piperacillin/tazobactam	65/73 (89%)	66/77 (86%)	133/150 (89%)
Resistant to fluoroquinolones	62/71 (87%)	66/66 (100%)	128/137 (93%)
Resistant to aminoglycosides	41/72 (57%)	13/66 (20%)	54/138 (39%)

SD, standard deviation.
[a] Any infection with *Klebsiella* spp. between days 1 and 180 after surgery.
[b] No infection with *Klebsiella* spp. between days 1 and 180 after surgery.
[c] Based on the applied weights, see Supplementary methods.
[d] Details are provided in Supplementary Table S9.
[e] In over 98% of screened patients, colonization screening was performed using a rectal swab.
[f] Observed colonization status; percentage among patients who underwent colonization screening, resistance percentage among patients who underwent resistance testing for the specific antibiotic.

number of eligible surgical procedures from which patients were selected and the number of eligible surgical procedures with a positive klebsiella culture.

Sample size

We aimed to include 1000 patients with and 1000 patients without a positive klebsiella clinical culture. With approximately 20 candidate predictor variables, this comprises up to 50 patients with an endpoint per predictor. Considering the sampling design, this is adequate for risk prediction studies and also enables a robust analysis of secondary endpoints [16]. The ratio of patients with and without a positive clinical culture was set to 1:1 to maximize the overall efficiency of the study.

Statistical analysis

As appropriate, results are presented as mean (± standard deviation (SD)) or median (± interquartile range (IQR)). We employed survey-weighted generalized linear models to enable calculation of absolute incidences [17]. Patients with a positive clinical culture received a weight of 1, while the weight of patients without a positive clinical culture was calculated as the inverse of the site-specific sampling fraction. The weighting deviated slightly from the protocol (see Supplementary methods). We analysed the independent predictive value of determinants for postsurgical *Klebsiella* spp. infection via multi-variable logistic regression. Model performance was assessed via calibration plots and the

Table II
Distribution of infecting pathogens, type of infection and site of infection among patients with at least one klebsiella infection

Category	Number of infected patients[a]	Percentage of infected patients[a]
Overall	840	100.0%
By *Klebsiella* species		
Klebsiella pneumoniae	720	85.7%
Klebsiella oxytoca	98	11.7%
Klebsiella aerogenes	33	3.9%
Klebsiella variicola	12	1.4%
Klebsiella, undifferentiated	18	2.1%
By antibiotic susceptibility[b]		
Resistant to 2nd/3rd generation cephalosporins	458/822	55.7%
Resistant to 4th generation cephalosporins	394/775	50.8%
Resistant to carbapenems	243/827	29.4%
Resistant to piperacillin/tazobactam	424/822	51.6%
Resistant to fluoroquinolones	399/822	48.5%
Resistant to aminoglycosides	234/822	28.4%
By type of infection		
Monobacterial infection	435	51.8%
Polybacterial infection	479	57.0%
By site of infection		
Surgical site infections (SSI)	459	54.6%
Superficial incisional SSI	107	12.7%
Deep incisional SSI	50	6.0%
Organ/space SSI	317	37.7%
Device-associated infections	279	33.2%
Catheter-associated urinary tract infections (CAUTI)	134	16.0%
Central line-associated blood stream infections (CLABSI)	102	12.1%
Ventilator-associated pneumonia	78	9.3%
Bloodstream infections (not including CLABSI)	184	21.9%
Primary bloodstream infections	31	3.7%
Secondary bloodstream infections	153	18.2%
Urinary tract infections (not including CAUTI)	101	12.0%
Pneumonia (non-ventilator associated)	53	6.3%
Other infections	24	2.9%

[a] Patients may have multiple infections from different categories; numbers and percentages do not add up.
[b] Percentage calculated among patients who underwent resistance testing for the antibiotic.

C-statistic. We employed internal cross-validation through bootstrapping with 1,000 iterations to recalibrate the model.

We conducted an additional unplanned sensitivity analysis to assess the impact of variation across hospitals in surgery type distribution, klebsiella colonization prevalence, and the incidence of postoperative klebsiella infection. This involved fitting the multi-variable model to six separate datasets, each time excluding one of the sites.

To explore trial enrichment potential, we calculated the conditional binomial sample size at various conveniently selected breakpoints of the predicted risk, with an assumed vaccine efficacy of 60%, a two-sided alpha of 0.05, and a power of 90%. This analysis was performed using the case-mix in three hospitals that included a broad range of surgery types.

Missing data

We imputed missing values using the Fully Conditional Specification method [18]. A custom imputation model was designed for the presumed klebsiella colonization status, accounting for variations in hospital detection methods. Two sites used MacConkey agars without any antibiotics. Two other sites used extended-spectrum β-lactamase (ESBL)-selective media, which we assumed to capture 40% of klebsiella colonization relative to MacConkey plates. Carbapenem-selective media, used by two sites, were assumed to identify 10% of klebsiella colonization relative to MacConkey plates. The assumed detection rates were based on the estimated prevalence of ESBL and carbapenem resistance within our study cohort. To account for the uncertainty in culture sensitivities between different culture media, we performed two sensitivity analyses with adjusted sensitivities of the ESBL and carbapenem-selective media: two times higher and two times lower compared with the main analysis. We employed lasso penalization for the imputation of presumed klebsiella colonization status to avoid overfitting [19]. Based on the quadratic rule, 175 imputations were needed [20]. For pragmatic reasons, we performed 200 imputations.

Ethical approval

A local medical ethical committee waiver for informed consent or approval of a no-objection policy was obtained for each participating site.

Table III
Multi-variable model for prediction of infection with klebsiella within 180 days after elective surgery

Variable	Crude			Bias-corrected	
	Coefficient	SE	OR (95% CI)	Coefficient	OR (95% CI)
(Intercept)	−3.9809	0.2032	–	−3.8384	–
Age (per year)	0.0011	0.0119	1.00 (0.98–1.02)	0.0009	1.00 (0.98–1.02)
Female sex	0.1176	0.1997	1.12 (0.76–1.66)	0.0930	1.10 (0.74–1.62)
Cardiovascular disease	0.7726	0.2177	2.17 (1.41–3.32)	0.6110	1.84 (1.20–2.82)
Chronic pulmonary disease	0.0586	0.3720	1.06 (0.51–2.20)	0.0463	1.05 (0.51–2.17)
Diabetes mellitus	0.1086	0.2354	1.11 (0.70–1.77)	0.0859	1.09 (0.69–1.73)
Moderate to severe chronic renal failure	0.5966	0.4321	1.82 (0.78–4.24)	0.4719	1.60 (0.69–3.74)
Solid organ malignancy	−0.4507	0.3234	0.64 (0.34–1.20)	−0.3564	0.70 (0.37–1.32)
Urological diseases including prostatitis	0.3794	0.3870	1.46 (0.68–3.12)	0.3000	1.35 (0.63–2.88)
Immune suppression	0.0850	0.3059	1.09 (0.60–1.98)	0.0672	1.07 (0.59–1.95)
Indwelling urinary catheter	1.1899	0.6788	3.29 (0.87–12.43)	0.9411	2.56 (0.68–9.69)
Harmful use of alcohol or alcohol dependence	2.0801	0.9569	8.01 (1.23–52.23)	1.6451	5.18 (0.79–33.81)
Non-prophylactic antibiotic treatment within 30 days before surgery	0.6199	0.4861	1.86 (0.72–4.82)	0.4903	1.63 (0.63–4.23)
Surgery within 90 days before the current surgery	0.7690	0.3739	2.16 (1.04–4.49)	0.6082	1.84 (0.88–3.82)
Cardiothoracic surgery	0.5126	0.3849	1.67 (0.79–3.55)	0.4054	1.50 (0.71–3.19)
Gastrointestinal surgery	2.3304	0.4646	10.28 (4.14–25.56)	1.8430	6.32 (2.54–15.70)
Orthopaedic surgery	−0.1636	0.4647	0.85 (0.34–2.11)	−0.1294	0.88 (0.35–2.18)
Other abdominal surgery	1.6859	0.4458	5.40 (2.25–12.93)	1.3334	3.79 (1.58–9.09)
Urological surgery	1.0061	0.6056	2.74 (0.83–8.96)	0.7957	2.22 (0.68–7.26)
Oncological surgery	0.0570	0.3081	1.06 (0.58–1.94)	0.0451	1.05 (0.57–1.91)
Trauma surgery	1.9511	0.5674	7.04 (2.31–21.39)	1.5431	4.68 (1.54–14.23)
Minimally invasive surgery	−1.4342	0.2925	0.24 (0.13–0.42)	−1.1343	0.32 (0.18–0.57)
Peri-operative prophylactic antibiotic treatment	−2.4094	0.6496	0.09 (0.03–0.32)	−1.9055	0.15 (0.04–0.53)
Colonized with *Klebsiella* spp.[a]	3.1219	0.6067	22.69 (6.91–74.52)	2.4690	11.81 (3.60–38.79)

CI, confidence interval; OR, odds ratio; SE, standard error.
[a] Presumed 'true' colonization based on the imputation model.

Results

A total of 139,778 elective surgeries met the inclusion criteria, with 2604 having postoperative klebsiella-positive clinical cultures. We included 1988 patients: 998 patients with a positive postsurgical clinical klebsiella culture and 990 without (Figure 1). One participating centre identified significantly more patients with a positive postsurgical culture ($N = 2077$) than the other centres. To ensure balanced representation across sites, all patients with a positive klebsiella culture were included, except at the largest centre, where only a random selection ($N = 471$) of patients with a positive postsurgical culture was included. After excluding transplant patients, 875 patients with and 906 without a postoperative klebsiella-positive culture were analysed. Among the 875 patients with positive cultures, 35 did not meet the CDC-NHSN infection criteria and were analysed in the group without klebsiella infection, resulting in 840 infected and 941 non-infected patients. One site included only transplant surgery patients; hence, six sites contributed data to the current analysis. One hospital included only gastrointestinal surgeries (particularly colorectal surgery); one included gastrointestinal and other abdominal surgeries (specifically pancreatic surgery); and one hospital included gastrointestinal, other abdominal, urological, and obstetric and gynaecological surgeries. Three sites included a broad range of surgery types. Applicable infection prevention measures per site are provided in Supplementary Table S1. Baseline characteristics are presented in Table I. Untargeted peri-operative antibiotic prophylaxis was administered in almost all surgeries, including those with klebsiella colonization. Of 95 observed colonized patients, 12 received adequate prophylaxis (including one control patient), 62 inadequate prophylaxis (two control patients), and five received no prophylaxis. In 16 cases, adequacy was unknown due to missing susceptibility data (one control patient).

Klebsiella infections

Klebsiella infection criteria were met in 1231 infection episodes among 840 patients, resulting in a 180-day cumulative incidence of first klebsiella infection of 1.38% (95% confidence interval (CI) 1.24–1.54). A total of 808 postsurgical infections occurred within 30 days post-surgery in 653 patients (78%), corresponding to a 30-day cumulative incidence of 1.07% (95% CI 0.96–1.20). *Klebsiella pneumoniae* was the most frequent causative pathogen (Table II). Surgical site infections were most common (54.6% of infected patients), followed by device-associated infections (33.2%) and bloodstream infections (21.9%). A total of 587 polybacterial infections occurred in 479 patients, with *Escherichia coli* (140, 24% of polybacterial infections), *Pseudomonas aeruginosa* (125, 21%), and *Enterococcus faecalis* (102, 17%) being common co-pathogens (Supplementary Table S2). The culture specimens associated with each infection type are detailed in Supplementary Table S3.

Multi-variable model

The strongest risk factor for klebsiella infection was pre-operative colonization with klebsiella (corrected odds ratio (OR) 11.8, 95% CI 3.6—38.8; Table III). Gastrointestinal surgery, other abdominal surgery, trauma surgery, and chronic cardiovascular disease were also significant independent predictors of higher risk, while minimally invasive surgery and peri-operative antibiotic prophylaxis were significantly associated with a lower risk of klebsiella infection. The corrected C-statistic for this model was 0.899 (95% CI 0.871—0.921). Model calibration (Figure 2a, b) was good for patients with a low predicted risk but poor for patients with a high predicted risk (i.e., a predicted risk >10%, which comprised <1% of the weighted population; see Figure 2c, d).

Sensitivity analyses

The planned sensitivity analyses, in which we varied the assumed sensitivity of selective screening techniques, confirmed colonization with klebsiella as a strong independent predictor (Supplementary Tables S4—S7). The effect of colonization was robust, yielding similar odds ratios and C-statistics. Using observed rather than imputed colonization status yielded a similar OR for klebsiella colonization but resulted in a significantly lower discriminative capacity of the model (corrected C-statistic 0.797, 95% CI 0.763—0.828), which was due to a lower prevalence of klebsiella colonization. In the unplanned sensitivity analysis, wherein we systematically excluded individual sites from the analysis one at a time, C-statistics remained consistent after each site exclusion, except for the

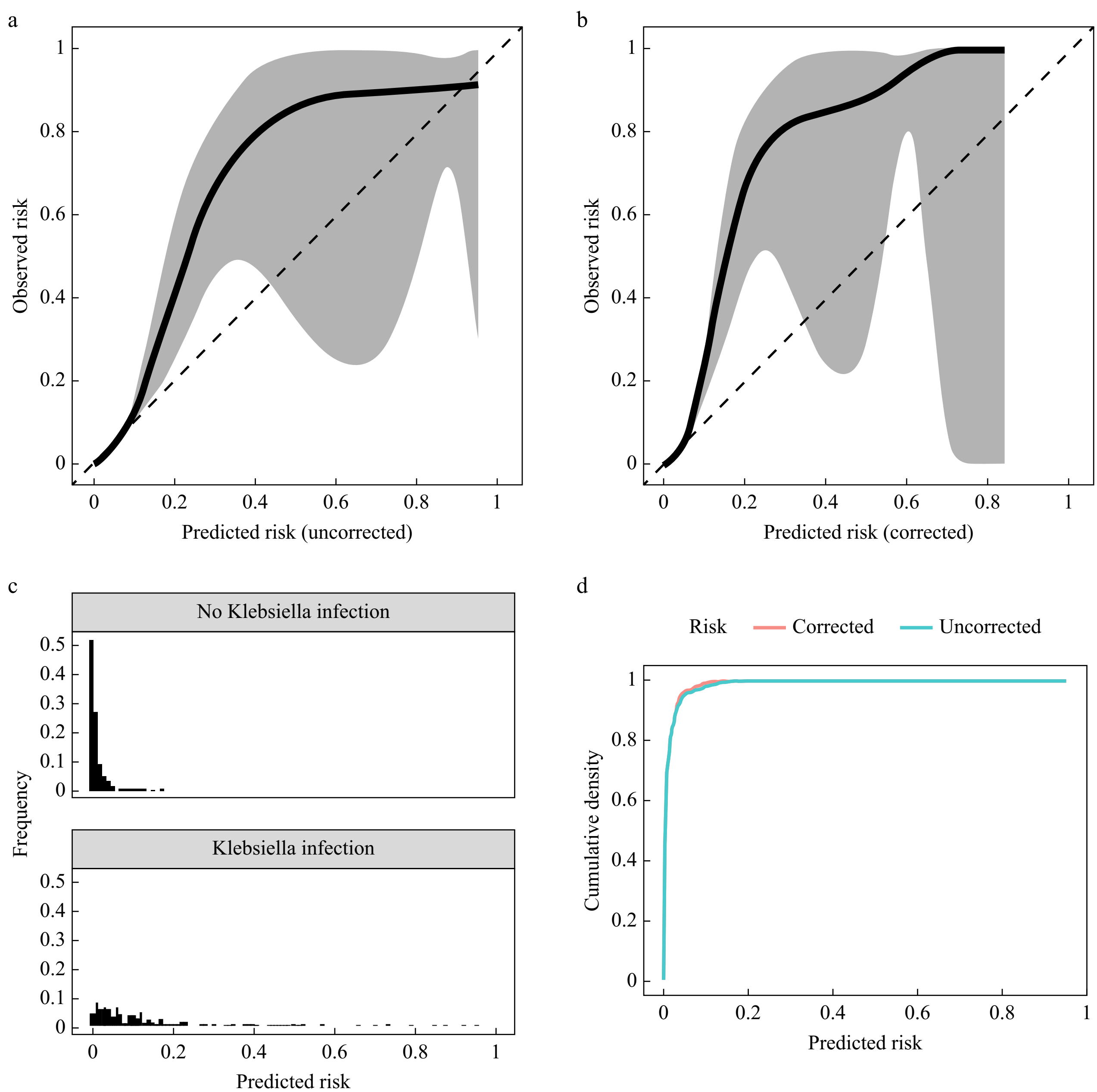

Figure 2. Performance of the multi-variable model.

largest site, which resulted in a decreased C-statistic of 0.856 (95% CI 0.811–0.892; Supplementary Table S8).

Trial enrichment potential

Trial enrichment simulation results are displayed in Table IV. Including all patients meeting the postoperative risk of infection with klebsiella in adults (PIKA) inclusion criteria (breakpoint of 0%) with an assumed vaccine efficacy of 60% would require 6720 patients in a randomized clinical trial. Restricting inclusion criteria to patients with a predicted risk >2.0% reduced the required number of trial participants to 1884 (72% reduction). Restricting inclusion to patients with either positive klebsiella colonization cultures or those undergoing gastrointestinal or other abdominal surgery resulted in a trial size reduction to 3186 (53% reduction). While the model efficiently reduces trial size, adopting trial enrichment strategies introduces a trade-off, necessitating an increase in the number of trial sites and/or extending the enrolment period to achieve the required sample size, due to some outcomes occurring in the excluded population. As a result, the optimal breakpoint for trial enrichment depends on trial-specific characteristics such as the availability of sites and the costs of opening additional sites. Notably, changing the assumed vaccine efficacy to, for example, 40% or 80% resulted in an almost identical relative reduction in trial size and increase in site years.

Discussion

The cumulative incidence of postsurgical klebsiella infection during the 180 days following elective inpatient surgery was 1.38% (95% CI 1.24–1.54%). Pre-surgical colonization with *Klebsiella* spp., gastrointestinal surgery, other abdominal surgery, trauma surgery and chronic cardiovascular disease were independent predictors of higher risk. Minimally invasive surgery and peri-operative antibiotic prophylaxis were independent predictors of lower risk. The predictors listed in Table III can be used to efficiently reduce the required sample size for future klebsiella infection prevention trials.

The study's main strengths are its large sample size and multi-centric nature, enhancing generalizability and providing robust insights into the incidence and distribution of klebsiella infections in this population. However, the study also has its limitations. Due to the study's retrospective nature and the heterogeneity between centres, selection and information bias may play a role (e.g., the retrospective adjudication of infections). A main vulnerability lies in the various screening methods and microbiological lab protocols used in the participating hospitals (selective vs non-selective media). We corrected for this by employing an advanced imputation model. Although the imputation model relied on assumed culture sensitivities, the robustness of the imputation model was affirmed through extensive sensitivity analyses in which we modelled varying sensitivities of the colonization media. The calibration of the prediction model was poor for patients at high risk for klebsiella infection (underprediction), even after internal validation. However, it should be noted that 99.1% of the weighted population had a predicted risk below 10% (Figure 2c, d). In practice, the underprediction of high-risk patients will make little difference, as the threshold for selecting patients in a future trial is far below the region where the predicted risk is underestimated. Finally, one of the included hospitals (Site 1 in Supplementary Table S2) reported that none of the enrolled patients exhibited a positive screening culture, because patients with a positive screening culture did not undergo elective surgery at this site. This could have led to an underestimation of the overall incidence of klebsiella infection in this study. To account for (unmeasured) variability between the centres, we performed a sensitivity analysis in which we excluded individual sites from the analysis. Excluding this site yielded a similar performance of the model. Therefore, differences in incidence are likely related to case mix differences captured in the model, most notably colonization prevalence.

No prior studies have systematically evaluated risk factors for klebsiella infection within a surgical population.

Table IV
Simulation of different scenarios for trial enrichment

Breakpoint	Proportion of PIKA cohort above breakpoint	Risk (obs)[a]	Risk 1 (pred)[b]	Risk 2 (pred)[c]	*N* events	*N* total	Change in trial size	Change in site, years[d]
At an assumed vaccine efficacy of 60%								
0.00%[e]	1.000	0.018	0.012	0.005	56	6720	–	–
0.25%	0.942	0.019	0.013	0.005	56	6386	−5.0%	+0.9%
0.50%	0.636	0.028	0.017	0.007	56	4774	−29.0%	+11.7%
1.00%	0.305	0.055	0.027	0.011	56	2938	−56.3%	+43.2%
2.00%	0.137	0.108	0.042	0.017	56	1884	−72.0%	+104.8%
Rule1[f]	0.360	0.038	0.025	0.010	56	3186	−52.6%	+31.6%
Rule2[g]	0.047	0.172	0.089	0.036	56	900	−86.6%	+185.6%
Rule3[h]	0.328	0.031	0.022	0.009	56	3674	−45.3%	+66.8%

PIKA, postoperative risk of infection with klebsiella in adults.
[a] Observed risk of klebsiella infection in the study population.
[b] Predicted risk of klebsiella infection in the control group.
[c] Predicted risk of klebsiella infection in the vaccine group.
[d] Calculated as ('*N* total'/'proportion above breakpoint'/6720−1) * 100%.
[e] Including all patients meeting PIKA inclusion criteria without further risk stratification.
[f] Colonized with klebsiella or undergoing gastrointestinal or other abdominal surgery.
[g] Colonized with klebsiella.
[h] Undergoing gastro-intestinal or other abdominal surgery.

Nevertheless, our findings align with expectations, highlighting colonization as a primary risk factor for klebsiella infection. This is consistent with observations from numerous studies in hospital settings, in which klebsiella strains identified through colonization screening were also cultured from infection sites [3,4,21,22]. Although the risk factors in our study are well-known risk factors for infection in general, our study provides a precise quantification of the risk of klebsiella colonization on postoperative infection. Because carriage of klebsiella is mainly manifested in the gastrointestinal tract, it is unsurprising that we identified gastrointestinal and other abdominal surgery as risk factors for postsurgical klebsiella infection, while we found a decreased risk after minimally invasive surgery [2]. We did not find diabetes or immune suppression to be significantly associated with postsurgical klebsiella infection, whereas previous studies did find an association [23–25]. However, this association was only observed in community-onset klebsiella infections [23].

By evaluating trial enrichment scenarios for a phase-III klebsiella infection prevention trial in elective surgery patients, this study provides the foundation for more efficient, targeted clinical trials. Given the low incidence of klebsiella infections in this population, achieving feasible sample sizes is challenging. Our trial enrichment simulation with different prediction rules and thresholds provides a comprehensive starting point for refining trial populations. Yet, future studies should validate the current prediction model in different contexts. An important consideration is that over half of the infections in this study were polybacterial. It is uncertain whether preventive interventions targeted against *Klebsiella* spp. only, can prevent these infections or reduce their severity. When planning a trial, anticipated effect sizes may need to be downsized accordingly.

In conclusion, the cumulative 180-day incidence of postsurgical klebsiella infection is 1.38%. Our multi-variable model integrating both clinical factors and klebsiella colonization status enabled efficient identification of patients at high risk for klebsiella infection in an elective surgery population, which can optimize the design of future infection prevention trials. By improving risk stratification, this model may enhance the feasibility and efficiency of phase III trials for preventive interventions, such as vaccines, ultimately contributing to targeted strategies against klebsiella infections.

Acknowledgements

We extend our sincere appreciation to Jonneke van der Beek, Eva Haanen, Esmée Arents, and Evelien Leffelaar for their contributions as part of the operational team at Ecraid and data management at the UMCU, as well as to Patricia Martin, Anne Tillè and Isabella Alberini for their contribution in the study design and follow-up as part of the clinical team at LimmaTech Biologics. Their dedicated efforts were vital to the success of this project. Additionally, we thank Professor Peter Hermans and Professor Marc Bonten for their roles in the conceptualization and realization of this study. We extend our thanks to Dr Valentina Galfo and Dr Lorenzo Nigi for their contributions to the screening and data collection at the University of Pisa. Furthermore, the authors would like to thank Dr Enrico Gibbin, Dr Daniele Fasan, Dr Martina Maruccio, and Dr Matteo De Pastena for their contributions to gathering the data at the University of Verona. Special recognition is extended to Professor Cristina Mussini for supervision, Giulia Burastero, Martina Del Monte, Alice Mazzocchi, and Irene Baldisserotto for their contributions to data collection at the University of Modena. We sincerely thank Professor Pasquale Stefanizzi for supervision, and Dr Antonio Di Lorenzo, for the data collection at the University of Bari. Additionally, we express our gratitude to Professor Massimo Puoti for supervision and Dr Federico D'Amico for study management and data collection from ASST Grande Ospedale Metropolitano Niguarda, Milan. We acknowledge the contribution of Elias Iosifidis for his valuable local supervision and advice, and Argyro Ftergioti and George Papacharalampous's dedicated contributions to data collection at Hippokration Hospital of Thessaloniki.

Author contributions

J.L.G.H.M. was involved in central medical monitoring and data analysis and wrote the first version of the manuscript. M.F., G.T., A.M.A., E.T., E.F., S.T., M.M., B.C., and E.R. were involved in patient selection and data collection. N.L.J.S. coordinated centralized operational activities. D.B. and V.V. contributed to the study protocol, statistical analysis plan, and interpretation of the data. C.H.W. designed the study, coordinated protocol and statistical analysis plan writing, study execution, and data analysis, and supervised manuscript writing. C.A. conceptualized the study, revised the study protocol, secured funding, supervised the study's progress, and contributed to the interpretation of the data. All authors critically reviewed the manuscript and took responsibility for its content.

Conflict of interest statement

M.F. declares research funding from Gilead Sciences to his institution and speaker fees from Pfizer, Menarini, GSK and ThermoFisher. G.T. has received speaker honoraria from Shionogi and Menarini, a congress attendance fee from Menarini and a fee for participation in the scientific board of MSD. S.T. declares grants from MSD, Sequirus and GSK, a speaker fee from Pfizer, travel fees from Sanofi Pasteur MSD and GSK and a fiduciary role in the Italian Society of Hygiene. M.M. declares research funding from Gilead Sciences to his institution and a travel fee from Pfizer Inc. E.R. has received research grants from Merck, AbbVie, Shionogi, Cidara Therapeutics and Pfizer Inc., to his institution, and is a scientific advisor and member of the speaker bureaux for Gilead, Merck, Shionogi, Mundipharma and Pfizer Inc. D.B. and V.V. declare their employment as staff members of GlaxoSmithKline and ownership of GlaxoSmithKline shares. C.H.W. declares research funding from DaVolterra and bioMérieux to his institution and consultancy fees from Merck/MSD and Sanofi-Pasteur to his institution. C.A. declares employment by LimmaTech Biologics. All other authors declare no additional conflicts of interest.

Funding sources

This study was funded by LimmaTech Biologics AG. All participating medical institutions received funding to conduct the study. The UMC Utrecht was the sponsor of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2025.04.036.

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