Clinical Microbiology and Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study

Wouter C. Rottier [1, *, †], J.W. Timotëus Deelen [1, †], Giorgia Caruana [1, 2, ‡], Anton G.M. Buiting [3], J. Wendelien Dorigo-Zetsma [4], Jan A.J.W. Kluytmans [5], Paul D. van der Linden [6], Steven F.T. Thijsen [7], Bart J.M. Vlaminckx [8], Annemarie J.L. Weersink [9], Heidi S.M. Ammerlaan [1, 10], Marc J.M. Bonten [1, 11]

[1] *Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands*
[2] *Unit of Infectious Diseases, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy*
[3] *Laboratory for Medical Microbiology and Immunology, Elisabeth-TweeSteden Hospital, Tilburg/Waalwijk, the Netherlands*
[4] *Central Laboratory for Bacteriology and Serology, Tergooi Hospitals, Hilversum, the Netherlands*
[5] *Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, the Netherlands*
[6] *Department of Clinical Pharmacy, Tergooi Hospitals, Hilversum, the Netherlands*
[7] *Department of Medical Microbiology and Immunology, Diakonessenhuis, Utrecht, the Netherlands*
[8] *Department of Medical Microbiology and Immunology, St. Antonius Hospital, Utrecht/Nieuwegein, the Netherlands*
[9] *Laboratory for Medical Microbiology and Immunology, Meander Medical Center, Amersfoort, the Netherlands*
[10] *Department of Internal Medicine, Catharina Hospital, Eindhoven, the Netherlands*
[11] *Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands*

ARTICLE INFO

Article history:
Received 29 March 2020
Received in revised form
5 July 2020
Accepted 10 July 2020
Available online xxx

Editor: L. Leibovici

Keywords:
Bacteraemia
Burden of antimicrobial resistance
Enterobacterales
Extended-spectrum beta-lactamase
Nonbacteraemic infection

ABSTRACT

Objectives: Antibiotic resistance in Gram-negative bacteria has been associated with increased mortality. This was demonstrated mostly for third-generation cephalosporin-resistant (3GC-R) Enterobacterales bacteraemia in international studies. Yet, the burden of resistance specifically in the Netherlands and created by all types of Gram-negative infection has not been quantified. We therefore investigated the attributable mortality of antibiotic resistance in Gram-negative infections in the Netherlands.

Methods: In eight hospitals, a sample of Gram-negative infections was identified between 2013 and 2016, and separated into resistant and susceptible infection cohorts. Both cohorts were matched 1:1 to non-infected control patients on hospital, length of stay at infection onset, and age. In this parallel matched cohort set-up, 30-day mortality was compared between infected and non-infected patients. The impact of resistance was then assessed by dividing the two separate risk ratios (RRs) for mortality attributable to Gram-negative infection.

Results: We identified 1954 Gram-negative infections, of which 1190 (61%) involved *Escherichia coli*, 210 (11%) *Pseudomonas aeruginosa*, and 758 (39%) bacteraemia. Resistant Gram-negatives caused 243 infections (12%; 189 (78%) 3GC-R Enterobacterales, nine (4%) multidrug-resistant *P. aeruginosa*, no carbapenemase-producing Enterobacterales). Subsequently, we matched 1941 non-infected controls. After adjustment, point estimates for RRs comparing mortality between infections and controls were similarly higher than 1 in case of resistant infections and susceptible infections (1.42 (95% confidence interval 0.66–3.09) and 1.32 (1.06–1.65), respectively). By dividing these, the RR reflecting attributable mortality of resistance was calculated as 1.08 (0.48–2.41).

Conclusions: In the Netherlands, antibiotic resistance did not increase 30-day mortality in Gram-negative infections. **Wouter C. Rottier, Clin Microbiol Infect 2020;▪:1**

* Corresponding author. W.C. Rottier, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Huispostnummer STR 6.131, Postbus 85500, 3508 GA, Utrecht, the Netherlands. Tel: +31 88 75 576 76; fax: +31 88 75 680 99.
E-mail address: w.c.rottier-2@umcutrecht.nl (W.C. Rottier).
† These authors contributed equally to this work.
‡ Present address: Institute of Microbiology, Department of Laboratory Medicine and Microbiology, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, Lausanne, Switzerland.

https://doi.org/10.1016/j.cmi.2020.07.014
1198-743X/

Introduction

The dissemination of resistant Gram-negative bacteria has become a major public health concern over the last decades. In the Netherlands, the most prevalent problem is third-generation cephalosporin (3GC) resistance among Enterobacterales, generally resulting from extended-spectrum β-lactamase (ESBL) production [1,2]. Outbreaks of carbapenemase-producing bacteria occur sporadically, mostly in hospitals after unnoticed introduction from abroad [3]. Dutch infection prevention guidelines define several Gram-negative highly resistant micro-organisms (HRMOs), for which targeted control measures are recommended to limit spread in healthcare settings (Table 1) [4].

This policy poses a large burden on resources, personnel and patients [5]. It is justified by the perceived additional disease burden of infections caused by resistant Gram-negatives, often narrowed down to the increase in mortality that can be causally related to antibiotic resistance, i.e. the attributable mortality. Quantifications thereof naturally stem from observational studies, which are hampered by confounding bias. To reduce residual confounding, De Kraker et al. proposed the parallel matched cohort design, in which both patients infected with resistant pathogens and patients infected with susceptible pathogens are compared with their own non-infected controls [6,7]. Their study, performed in 13 European countries but not in the Netherlands, yielded an odds ratio (OR) of 2.5 (95% confidence interval (CI) 0.9–6.8) when comparing 30-day mortality between bacteraemia caused by 3GC-resistant and -susceptible *Escherichia coli* [7].

Yet, as only patients with bacteraemia were studied, it remained unknown how resistance impacts non-bacteraemic infections, reflecting the majority of infections, and to what extent these findings reflected the situation in the Netherlands. Therefore, we studied the attributable mortality of HRMO Gram-negative infections in a parallel matched cohort in Dutch hospitals.

Methods

Study design, setting and participants

We aimed to enrol a representative sample of 2000 patients with Gram-negative (specified in Table 1) infection from eight Dutch hospitals, including one university hospital (Supplementary Table S1). At each participating site, five infection episodes were included weekly, over the course of approximately 1 year between June 2013 and February 2016. We defined Gram-negative infections based on microbiological and clinical criteria as described by Horan et al. [8]. Enrolled patients had to be at least 18 years of age, infection episodes had to be associated with admission to a clinical acute care ward, and patients had to be treated with oral or intravenous antibiotics. An individual patient could be included with several infection episodes.

For each infection episode, a control patient from the same hospital with no evidence of Gram-negative infection was matched based on a similar length of stay in the same hospital on the day of infection onset, and similar age. For community-onset infections, only emergency admissions were eligible for matching. A single patient could serve as the control patient for several infection episodes.

Considerations for the sample size, the weekly screening procedure, definitions of infection entities and index cultures, and the procedure for matching control patients are described in detail in the Supporting Information.

The institutional review board of the University Medical Centre Utrecht judged that the Dutch Medical Research Involving Human Subjects Act did not apply to this study, and a waiver for informed consent with regard to the information presented in this manuscript was obtained in all participating hospitals. This study formed part of a more extensive project named GRAND-ABC, of which the protocol is available as Supporting Information (registered at clinicaltrials.gov under number NCT02007343).

Data collection for exposure, outcomes and confounders

Infection onset was defined at the moment at which the first index culture was obtained. All pathogens obtained from index cultures (defined in the Supporting Information) were considered causative pathogens of the infection episodes, including, e.g., Gram-positive bacteria and yeasts. Based on antibiotic susceptibility testing [9], the Gram-negative isolates were categorized as HRMO or non-HRMO (Table 1). If at least one Gram-negative isolate constituted an HRMO, the infection was considered an HRMO infection. All others were categorized as non-HRMO infections. Intravenous and oral antibiotic therapy provided on the day of infection onset was categorized as appropriate or inappropriate based on the susceptibility of only the Gram-negative isolates in index cultures.

Table 1
Definition of Gram-negative highly resistant micro-organisms (HRMO)

Organism group	HRMO definition based on Dutch HRMO guidelines [4]
Enterobacterales[a]	(Ceftazidime R *OR* cefotaxime/ceftriaxone R)[b] *OR* meropenem R[c] *OR* (ciprofloxacin R *AND* (gentamicin R *OR* tobramycin R))
Pseudomonas aeruginosa	*3/5 from:* Piperacillin + tazobactam[d] R, ceftazidime R, meropenem R[c], (gentamicin R *OR* tobramycin R), ciprofloxacin R
Acinetobacter spp.	Meropenem R[c] *OR* (ciprofloxacin R *AND* (gentamicin R *OR* tobramycin R))
Stenotrophomonas maltophilia	Co-trimoxazole R

Resistance (R) is defined by applying to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [9] to minimum inhibitory concentrations obtained through automated systems (Vitek 2 (bioMérieux SA, Marcy l'Etoile, France) or Phoenix (BD, Franklin Lakes, NJ, USA)), and includes isolates categorized as intermediate to the antibiotic.

[a] In this study, Enterobacterales included *Citrobacter* spp., *Enterobacter* spp. (including *Enterobacter*/*Klebsiella aerogenes*, *Enterobacter*/*Kluyvera intermedia* and *Enterobacter*/*Cronobacter sakazakii*), *Escherichia* spp., *Hafnia* spp., *Klebsiella* spp. (including *Klebsiella*/*Calymmatobacterium granulomatis* and *Klebsiella*/*Raoultella* spp.), *Morganella* spp., *Pantoea* spp., *Proteus* spp., *Providencia* spp., and *Serratia* spp.

[b] Dutch HRMO guideline uses extended-spectrum beta-lactamase (ESBL) positive for this criterion.

[c] Dutch HRMO guideline uses carbapenemase-positive for this criterion.

[d] Dutch HRMO guideline uses piperacillin-resistant for this criterion.

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014

The primary outcome was all-cause mortality within 30 days after infection onset or day of matching, based on the nationwide Personal Record Database. Secondary outcomes were length of hospital stay (prespecified) and intensive care unit (ICU) stay after infection onset, discharge destination and infection resolution at 14 days after infection onset. Detailed definitions of all collected variables, including confounders, are provided in the Supporting Information.

Statistical analysis

All statistical analyses were performed in R (version 3.6.3) [10], with the use of packages *Hmisc* [11], *rms* [12], *mice* [13] and *xtable* [14]. Missing data was dealt with through multiple imputation (see Supporting Information).

Statistical modelling was performed with regard to 30-day mortality only. The primary analysis, the parallel-cohorts analysis, started with the creation of two separate models: one comparing non-HRMO infections and one comparing HRMO infections to their respective non-infected controls. Then, a risk ratio (RR) for HRMO status was calculated by dividing the HRMO cohort-specific RR by the non-HRMO cohort-specific RR. This procedure was performed with and without adjustment for patient-related confounders. A secondary analysis, the infection-cohort analysis, was performed without reference to the matched non-infected patients. Again, models were created with and without adjustment for patient-related confounders, but additionally, infection-related variables (such as infection type, pathogen and sepsis severity) were added to evaluate their mediation of any relation between HRMO status and mortality. Furthermore, models were created to analyse the attributable 30-day mortality of (a) inappropriate antibiotic therapy provided on the day of infection onset, and (b) acquisition of a hospital-onset Gram-negative infection (HRMO or non-HRMO). The Supporting Information provides a description of all modelling details and post hoc subgroup analyses, involving restrictions to subgroups of infections and alternative definitions of resistance.

Results

Study patients

The sampling process resulted in inclusion of 1954 Gram-negative infection episodes (Fig. 1, Table 2). Most infections involved *E. coli* ($n = 1190$, 61%), whereas *Pseudomonas aeruginosa* was cultured in 210 episodes (11%), and 292 episodes (15%) involved more than one Gram-negative species (Table 3). At least one HRMO was identified in 243 (12%) infections, mostly 3GC-resistant Enterobacterales ($n = 189$, 78%). In six instances, carbapenem-resistant Gram-negatives were involved, none of which were carbapenemase-producing Enterobacterales. Bacteraemia was present in 758 (39%) infections. Most infections had the urinary tract as source ($n = 1001$, 52%), and 72 (4%) infections were complicated by haematogenous spread, infection of prosthetic material, osteomyelitis, and/or endocarditis.

Patients with HRMO infections had more prior healthcare exposure (Table 4), and these infections less frequently involved bacteraemia (Table 3). Thirty-day mortality was 10% ($n = 25$) for HRMO and 11% ($n = 190$) for non-HRMO infections (Table 5). Proportions of patients receiving oral or intravenous therapy on the day of infection onset, and the day before, were comparable for HRMO and non-HRMO infections (Table 3). However, antibiotic therapy on the day of infection onset was inappropriate in 68% ($n = 142$) and 39% ($n = 567$) of HRMO and non-HRMO infections, respectively. Inappropriate antibiotic therapy on the day of infection onset was not associated with higher 30-day mortality (unadjusted RR 0.83, 95% CI 0.62–1.12; adjusted RR 0.79, 95% CI 0.58–1.07; Supplementary Table S6).

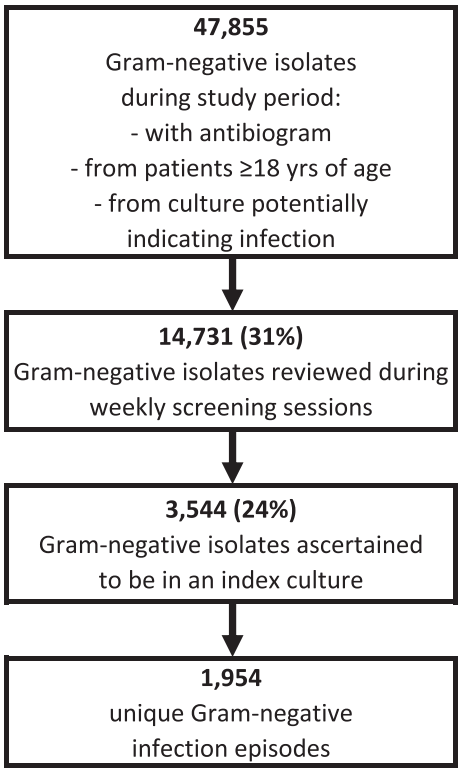

Fig. 1. Flow diagram of the screening process. During weekly screening sessions in eight Dutch hospitals, Gram-negative isolates newly reported by the local microbiology laboratory were consecutively reviewed until five Gram-negative infection episodes were identified for inclusion in the study. Screening took place between June 2013 and February 2016, during the course of approximately 1 year in each hospital (Supplementary Table S1). More details of the screening process are provided in the Supporting Information.

Matched non-infected control patients ($n = 1941$) had similar age and prior length of stay, but were admitted to different wards, had less comorbidity, and in general had less prior healthcare exposure than infected patients (Table 4). After the day of matching, their hospital stay was shorter (median 5 vs 8 days), and 30-day mortality was lower (8% ($n = 160$) vs 11% ($n = 215$); Table 5).

Attributable mortality

After full adjustment for confounding variables, the relative risks for 30-day mortality were 1.42 (95% CI 0.66–3.09) for HRMO infections and their non-infected controls, and 1.32 (95% CI 1.06–1.65) for non-HRMO infections and their non-infected controls (Fig. 2). Based on both RRs, the overall RR for 30-day mortality associated with HRMO status was 1.08 (95% CI 0.48–2.41).

When analysing infected patients only (i.e. without controls) the RR for 30-day mortality for HRMO infections was 0.78 (95% CI 0.50–1.21; Fig. 2) after adjustment for patient-related confounders, and 0.94 (95% CI 0.60–1.47) after further inclusion of infection-related mediators in the adjustment procedure (Supplementary Table S6). Hospital-onset Gram-negative infections (both HRMO and non-HRMO; $n = 554$) were, compared with their non-infected controls, associated with increased 30-day mortality (adjusted RR 1.58 with 95% CI 1.12–2.22; Supplementary Table S6). Sensitivity analyses all showed point estimates indicating a protective effect of HRMO infection on mortality (Supplementary Table S7).

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014

Table 2
Distribution of characteristics among all cultures, screened cultures and index cultures

	All relevant isolates during study period[a], *n* (%)	Bacterial isolates from screened cultures, *n* (%)	Bacterial isolates from index cultures, *n* (%)
Material			
Blood culture	4008 (8.38)	1519 (10.31)	1155 (32.59)
Urine	24,323 (50.83)	6845 (46.47)	1160 (32.73)
Lower respiratory tract	8079 (16.88)	2637 (17.90)	251 (7.08)
Fluid, pus, tissue (biopsy)	5505 (11.50)	1962 (13.32)	718 (20.26)
Swab	5186 (10.84)	1549 (10.52)	243 (6.86)
Other	754 (1.58)	219 (1.49)	17 (0.48)
Bacterial isolate			
Escherichia coli	22,145 (46.28)	6705 (45.52)	1904 (53.72)
Pseudomonas aeruginosa	5835 (12.19)	1916 (13.01)	337 (9.51)
Klebsiella pneumoniae	4426 (9.25)	1389 (9.43)	346 (9.76)
Proteus mirabilis	3609 (7.54)	1079 (7.32)	232 (6.55)
Enterobacter cloacae cx.	2587 (5.41)	801 (5.44)	177 (4.99)
Acinetobacter spp.	713 (1.49)	223 (1.51)	45 (1.27)
Stenotrophomonas maltophilia	647 (1.35)	189 (1.28)	20 (0.56)
Other	7893 (16.49)	2429 (16.49)	483 (13.63)
HRMO isolate	**6323 (13.21)**	**1972 (13.39)**	**390 (11.00)**
Enterobacterales: carbapenem resistant (±AG + FQ resistant)	90 (0.19)	26 (0.18)	2 (0.06)
Enterobacterales: 3GC resistant (±AG + FQ resistant)	4545 (9.50)	1435 (9.74)	300 (8.47)
Enterobacterales: AG + FQ resistant	1130 (2.36)	333 (2.26)	69 (1.95)
Pseudomonas aeruginosa: multidrug resistant	492 (1.03)	165 (1.12)	18 (0.51)
Acinetobacter spp.: carbapenem or AG + FQ resistant	41 (0.09)	12 (0.08)	1 (0.03)
Stenotrophomonas maltophilia: cotrimoxazole resistant	37 (0.08)	9 (0.06)	0 (0.00)
Total number of isolates	**47,855 (100.00)**	**14,731 (100.00)**	**3544 (100.00)**

Abbreviations: 3GC, third-generation cephalosporin; AG, aminoglycoside; FQ, fluoroquinolone; HRMO, highly resistant micro-organism.
[a] All Gram-negative isolates (listed in Table 1) with an antibiogram, from patients ≥18 years of age, from culture potentially indicating infection.

Discussion

In this study, we aimed to derive a cohort of patients with Gram-negative infections accurately reflecting patients with Gram-negative infections admitted in Dutch hospitals, as well as a matched cohort of non-infected control patients. Based on different methods for quantifying the association between antibiotic resistance and patient outcome we estimate that the attributable mortality of antibiotic resistance in this setting is close to zero, despite a 53% lower proportion of patients with infections caused by HRMOs receiving appropriate antibiotic therapy at the time of infection onset.

These findings are at odds with much of the prior literature on the attributable mortality of antibiotic resistance in Gram-negative infections. Importantly, in this study, highly effective definitive treatment remained available for infections caused by antibiotic-resistant bacteria, as only two infections were caused by strains combining carbapenem and fluoroquinolone resistance. As a result, antibiotic resistance reduces appropriateness of empiric therapy [15]. In settings with higher prevalence of infections caused by pathogens with complete β-lactam resistance in combination with fluoroquinolone resistance, both empiric and definitive therapy is less efficacious, which may differently impact patient outcome [16].

Our estimate mainly reflects the attributable mortality of ESBL-producing bacteria. Even in countries with more resistant bacteria, this estimate bears relevance for public health, as community-onset ESBL-producing *E. coli* infections greatly outnumber infections caused by carbapenem-resistant strains, which are mainly observed in hospital-acquired infections [17]. However, most estimates of attributable mortality due to multidrug-resistant, yet still treatable micro-organisms, differ from ours. For instance, two meta-analyses and two large European multicentre studies reported markedly increased mortality associated with ESBL-producing or 3GC-resistant Enterobacterales [7,18–20]. Our findings are more in line with other recent findings from a large multicentre study on Gram-negative bacteraemia, also reporting no impact of inappropriate initial therapy on outcome [21]. Others have also questioned the dogma of irreparable damage in case of inappropriate initial antibiotics in infections presenting without septic shock [22].

In theory, our finding of absence of attributable mortality due to infections caused by ESBL-producing Enterobacterales might be the result of local circumstances. Yet, we consider it unlikely that local bacterial epidemiology explains heterogeneity of attributable mortality as, to the best of our knowledge, the relevance of clones combining increased virulence and resistance to mortality rates in Gram-negative infection has not been convincingly demonstrated. Factors more likely to be implicated are local case-mix and practices of treating hospitalized patients, such as differences in turn-around times for antibiotic susceptibility results and the subsequent adaptation of inappropriate antibiotic therapy. The relevance of such heterogeneity should also be considered in country-specific calculations of annual numbers of deaths due to antibiotic resistance, as presented in a recent European study [17]. For the Netherlands, the estimate amounted to 206 deaths per year, of which 187 reportedly occurred in patients contracting Gram-

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014

Table 3
Characteristics of Gram-negative infection episodes

	Patients with non-HRMO infection, *n*/*N* with data (%)	Patients with HRMO infection, *n*/*N* with data (%)
Type of infection		
-Bacteraemia	680/1711 (40)	78/243 (32)
-Urinary tract infection	884/1696 (52)	117/240 (49)
-Respiratory tract infection	139/1696 (8)	19/240 (8)
-Intra-abdominal infection (excl. biliary tract)	199/1696 (12)	32/240 (13)
-Biliary tract infection	130/1696 (8)	18/240 (8)
-Skin/soft tissue/wound infection (incl. mediastinitis)	196/1696 (12)	38/240 (16)
-Other infection source	80/1696 (5)	11/240 (5)
-Postoperative infection	141/1711 (8)	28/243 (12)
Causative pathogens: Gram-negatives[a,b]		
-*Escherichia coli*	881/1711 (51)	116/243 (48)
-*Klebsiella pneumoniae*	132/1711 (8)	12/243 (5)
-*Enterobacter cloacae cx.*	49/1711 (3)	27/243 (11)
-*Proteus mirabilis*	96/1711 (6)	3/243 (1)
-*Pseudomonas aeruginosa*	135/1711 (8)	9/243 (4)
-Other Gram-negative[b] species	181/1711 (11)	21/243 (9)
-Multiple Gram-negative[b] species	237/1711 (14)	55/243 (23)
HRMO phenotype		
-Enterobacterales: carbapenem resistant (±AG + FQ resistant)		2/243 (1)[c]
-Enterobacterales: 3GC resistant (±AG + FQ resistant)		189/243 (78)
-Enterobacterales: AG + FQ resistant		47/243 (19)
-*Pseudomonas aeruginosa*: multidrug resistant		9/243 (4)[d]
-*Acinetobacter spp.*: carbapenem or AG + FQ resistant		1/243 (0)[e]
-*Stenotrophomonas maltophilia*: cotrimoxazole resistant		0/243 (0)
Causative pathogens: involvement of bacteria other than Gram-negatives[b], or yeast	456/1708 (27)	72/243 (30)
Sepsis severity[f] at infection onset[a]		
-No sepsis	512/1710 (30)	74/243 (30)
-Sepsis	963/1710 (56)	133/243 (55)
-Severe sepsis	115/1710 (7)	21/243 (9)
-Septic shock	120/1710 (7)	15/243 (6)
Antibiotic treatment during the infection episode		
-Receipt of antibiotic therapy prior to hospital admission	167/1710 (10)	30/243 (12)
-Receipt of antibiotic therapy[g] on the day prior to infection onset	249/1466[h] (17)	46/210[h] (22)
-Receipt of antibiotic therapy[g] on the day of infection onset	1176/1466[h] (80)	164/210[h] (78)
-Receipt of inappropriate antibiotic therapy[i] on the day of infection onset for Gram-negative causative pathogens	567/1466[h] (39)	142/210[h] (68)
Source control performed during the admission after infection onset	570/1711 (33)	94/243 (39)
Status of the infection episode at 14 days after infection onset[a]		
-Patient admitted − infection resolved	183/1711 (11)	38/243 (16)
-Patient admitted − mere completion of antibiotic course	60/1711 (4)	10/243 (4)
-Patient admitted − infection ongoing	150/1711 (9)	30/243 (12)
-Patient discharged − infection resolved at discharge	290/1711 (17)	55/243 (23)
-Patient discharged − mere completion of antibiotic course after discharge	810/1711 (47)	77/243 (32)
-Patient discharged − infection ongoing at discharge	103/1711 (6)	17/243 (7)
-Patient deceased	115/1711 (7)	16/243 (7)

3GC, third-generation cephalosporin; AG, aminoglycoside; FQ, fluoroquinolone; HRMO, highly resistant micro-organism.
[a] Mutually exclusive categories.
[b] Restricted to species listed in Table 1.
[c] One *E. coli*, and one *E. cloacae cx.* isolate without non-wild-type resistance to other beta-lactams or co-resistance, hence unlikely to represent true carbapenemase producers.
[d] Of which four carbapenem-resistant.
[e] Of which none carbapenem-resistant.
[f] According to Sepsis-2 criteria (see Supplementary Table S4).
[g] In-hospital prescriptions only.
[h] Available for seven of eight hospitals.
[i] In-hospital and post-discharge prescriptions. Includes receipt of no oral/intravenous antibiotic therapy.

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014

Table 4
Characteristics and outcomes of patients with Gram-negative infections and non-infected control patients

	Non-HRMO cohort		HRMO cohort	
	Non-infected control patients, *n/N* with data (%)	Patients with Gram-negative infection, *n/N* with data (%)	Non-infected control patients, *n/N* with data (%)	Patients with Gram-negative infection, *n/N* with data (%)
Patient-related confounders				
Female	845/1700 (50)	825/1711 (48)	116/241 (48)	90/243 (37)
Age, median (IQR)	72 (6281)	71 (61–80)	70 (60–77)	68 (60–77)
Other bacterial infection at infection onset	361/1700 (21)	137/1446 (9)	57/241 (24)	12/204 (6)
Known colonization with an HRMO	35/1700 (2)	74/1711 (4)	10/241 (4)	68/243 (28)
Gram–negative bacteraemia during the year prior to infection onset	14/1700 (1)	77/1711 (5)	6/241 (2)	22/243 (9)
Preceding hospital admission within 3 months prior to infection onset	373/1695 (22)	553/1710 (32)	55/241 (23)	90/243 (37)
Admission from long–term care facility	61/1699 (4)	90/1710 (5)	7/241 (3)	23/243 (9)
Admission type				
-Via emergency ward	1364/1700 (80)	1345/1711 (79)	185/241 (77)	178/243 (73)
-Other form of emergency admission	151/1700 (9)	159/1711 (9)	19/241 (8)	20/243 (8)
-Elective admission	131/1700 (8)	176/1711 (10)	27/241 (11)	36/243 (15)
-Transfer from other hospital	54/1700 (3)	31/1711 (2)	10/241 (4)	9/243 (4)
Origin of infection				
-Community–onset, not healthcare–associated	891/1687 (53)	660/1705 (39)	112/240 (47)	62/242 (26)
-Community–onset, possibly healthcare –associated	14/1687 (1)	59/1705 (3)	0/240 (0)	6/242 (2)
-Community–onset, healthcare–associated	319/1687 (19)	522/1705 (31)	36/240 (15)	84/242 (35)
-Hospital–onset	463/1687 (27)	464/1705 (27)	92/240 (38)	90/242 (37)
Length of hospital stay prior to infection onset in case of hospital–onset infection, median (IQR)	8 (5–14)	8 (5–14)	12 (6–21)	12 (7–26)
Hospital ward at infection onset				
-Emergency ward	793/1700 (47)	733/1711 (43)	100/241 (41)	83/243 (34)
-Internal medicine	197/1700 (12)	217/1711 (13)	32/241 (13)	35/243 (14)
-Surgery or gastro–enterology	280/1700 (16)	390/1711 (23)	46/241 (19)	79/243 (33)
-Urology	33/1700 (2)	91/1711 (5)	5/241 (2)	19/243 (8)
-Pulmonary medicine	92/1700 (5)	83/1711 (5)	10/241 (4)	11/243 (5)
-ICU	43/1700 (3)	67/1711 (4)	8/241 (3)	7/243 (3)
-Other ward	262/1700 (15)	130/1711 (8)	40/241 (17)	9/243 (4)
Charlson comorbidity index, median (IQR)	1 (0–3)	2 (0–3)	1 (0–3)	2 (1–4)
Immunodeficiency	145/1700 (9)	206/1710 (12)	23/241 (10)	31/243 (13)
Solid malignancy	335/1700 (20)	507/1711 (30)	44/241 (18)	75/243 (31)
Treatment restriction in place prior to infection onset	438/1699 (26)	423/1711 (25)	57/241 (24)	79/243 (33)
Surgical procedure during the 30 days prior to infection onset	251/1700 (15)	325/1486 (22)	36/241 (15)	57/218 (26)
ICU stay during the 30 days prior to infection onset	113/1700 (7)	133/1452 (9)	22/241 (9)	33/209 (16)
Receipt of prophylactic antibiotic therapy at hospital admission	44/1699 (3)	50/1711 (3)	5/240 (2)	16/243 (7)
Outcomes				
ICU stay during the admission from infection onset onwards				
-No	1580/1700 (93)	1476/1711 (86)	227/241 (94)	207/243 (85)
-Already in ICU for >12 h at infection onset	33/1700 (2)	42/1711 (2)	5/241 (2)	7/243 (3)
-Already in ICU for 0–12 h at infection onset	18/1700 (1)	36/1711 (2)	2/241 (1)	2/243 (1)
-Admission to ICU within 0–12 h after infection onset	26/1700 (2)	90/1711 (5)	4/241 (2)	18/243 (7)
-Admission to ICU >12 h after infection onset	43/1700 (3)	67/1711 (4)	3/241 (1)	9/243 (4)
-Length of hospital stay after infection onset, median (IQR)	5 (3–9)	8 (5–14)	6 (3–12)	9 (6–16)
Discharge destination				
-Home	1156/1700 (68)	993/1711 (58)	152/241 (63)	116/243 (48)
-Home with home healthcare	115/1700 (7)	255/1711 (15)	22/241 (9)	45/243 (19)
-Long-term care facility	259/1700 (15)	263/1711 (15)	46/241 (19)	50/243 (21)
-Terminal care	25/1700 (1)	36/1711 (2)	5/241 (2)	5/243 (2)
-Deceased during admission	81/1700 (5)	138/1711 (8)	6/241 (2)	21/243 (9)
-Other hospital	64/1700 (4)	26/1711 (2)	10/241 (4)	6/243 (2)
Gram-negative bacteraemia within 7–90 days after infection onset	20/1700 (1)	54/1711 (3)	3/241 (1)	10/243 (4)
All-cause mortality within 30 days after infection onset	145/1695 (9)	190/1709 (11)	15/241 (6)	25/243 (10)

In case of non-infected control patients, infection onset refers to the moment at which the matched infected patient has their infection onset. This point in time was also used to categorize the (fictitious) 'origin of infection' for control patients. HRMO, highly resistant micro-organism; ICU, intensive care unit; IQR, interquartile range.

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014

Table 5
All-cause 30-day mortality

	Non-HRMO cohort, *n/N* within stratum (%)	HRMO cohort, *n/N* within stratum (%)	All episodes, *n/N* within stratum (%)
Patients with Gram-negative infection			
Community-onset episode	116/1239 (9.4)	17/152 (11.2)	133/1391 (9.6)
Hospital-onset episode	73/464 (15.7)	8/90 (8.9)	81/554 (14.6)
All episodes	190/1709 (11.1)	25/243 (10.3)	215/1952 (11.0)
Non-infected control patients			
Community-onset episode	95/1220 (7.8)	8/148 (5.4)	103/1368 (7.5)
Hospital-onset episode	50/462 (10.8)	7/92 (7.6)	57/554 (10.3)
All episodes	145/1695 (8.6)	15/241 (6.2)	160/1936 (8.3)

In case of non-infected control patients, the distinction community-onset vs hospital-onset episode is based on the moment at which the matched infected patient has their infection onset. HRMO, highly resistant micro-organism.

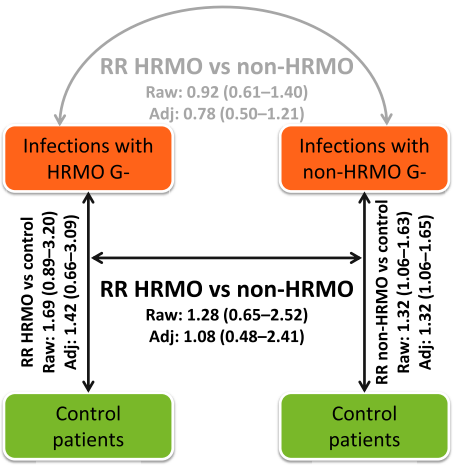

Fig. 2. Structure of the parallel matched cohort. This figure depicts the two methods applied to derive risk ratios (RRs) comparing highly resistant micro-organism (HRMO) to non-HRMO infections with regard to 30-day mortality. The elements of the parallel-cohorts analysis are shown in black, and the infection-cohort analysis is shown in grey. RRs are presented in their raw (unadjusted) form and after adjustment for patient-related confounders (listed in Supplementary Table S6). Derivation of the models is described in the Supporting Information. Adj, adjusted; G-, Gram-negative.

negative infections. These numbers resulted from using an attributable mortality factor derived from studies performed in settings not comparable to our country.

The absence of a discernible attributable mortality of resistant pathogens does not imply that there is no burden of disease imposed by these pathogens. Antibiotic-resistant pathogens may not just replace their antibiotic-susceptible counterparts, but their dissemination may in fact inflate the total number of infections [23,24]. Furthermore, this study aimed to estimate the contribution of resistance to mortality in the complete spectrum of Gram-negative infections encountered in Dutch hospitals, including mild infections. In fact, 61% of infections were non-bacteraemic, and 20% of patients did not receive antibiotic therapy on the day of infection onset. Our findings do therefore not exclude the possibility of attributable mortality due to resistance in subgroups of patients. Post hoc subgroup analyses did, however, not provide such indications for bacteraemic infections and patients with severe sepsis (Supplementary Table S7). Moreover, antibiotic resistance may also increase morbidity and costs. Indeed, we observed more healthcare exposure after infections caused by resistant bacteria (Table 4), but this may have been confounded by a pre-existing higher demand for healthcare in this group, and may not be attributable to antibiotic resistance.

The use of the parallel matched cohort design arguably reduces confounding in observational studies on the impact of antimicrobial resistance [6,7]. Interestingly, point estimates reflecting the attributable mortality of resistant bacteria differed considerably between the parallel-cohorts and infection-cohort analysis, although with largely overlapping confidence intervals (Fig. 2). More obviously, the design allows contrasting of the impact of resistance with the impact of infection. This is only possible for hospital-onset infections, as for community-onset infections (72% in this study), the most appropriate controls would be subjects from the open population instead of hospital controls. As such, we quantified that contracting a hospital-onset infection increases 30-day mortality by 58%, but that antibiotic resistance does not lead to a further increase (Supplementary Table S6).

A potential study limitation is that the cohort of infected patients was not a true random sample, as we included systematically five infected patients per week, whereas we generalized our findings to all Gram-negative infections occurring in Dutch hospitals. Furthermore, ICU-acquired pneumonia episodes may have been underrepresented, as sputum or tracheal aspirate from ICU patients were not considered as proof of infection. This was also motivated because of the current practice of routine use of Selective Digestive Decontamination in Dutch ICUs, which hampers the distinction between respiratory tract samples for surveillance or for clinical reasons.

A second potential limitation is that screening and selection of episodes may have been amenable to interobserver variability [25], and selective inclusion conditional on HRMO status may have occurred. For example, adjustment of antibiotic therapy to the susceptibility results was a prerequisite for some infections. Also, HRMO infections might represent selection of resistant flora in case of late diagnostic culturing, or patients under increased surveillance for the occurrence of infection could be overrepresented among HRMO infections. However, bacteraemia episodes would not be affected by these sampling issues and findings for this subgroup were very similar.

In conclusion, we find that antibiotic-resistant Gram-negatives for which effective second-line antibiotics are readily available do not increase 30-day mortality, which suggests that a delay in appropriate antibiotic therapy can be mitigated during the course of most infections. In the context of a high-income country with sporadically occurring carbapenem resistance, the total attributable mortality due to antibiotic resistance in Gram-negatives may be very low. Our findings emphasize the need for obtaining more refined estimates of attributable mortality, stratified on setting and

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014

treatment impact of resistance, in order to quantify the national and international burden of antibiotic resistance.

Transparency Declaration

The authors have no conflicts of interest to disclose. This work was supported by a research grant from the Netherlands Organisation for Health Research and Development (project number 205200007).

Acknowledgements

The authors would like to thank all research nurses for their involvement in data collection for this study, and all support staff at the participating sites for providing us with the opportunity to perform this study. Finally, we thank Henri van Werkhoven for critically reviewing an earlier version of this manuscript.

Results from this study were presented at the 27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria (22-25 April 2017, ePoster EV0541).

Author contributions

W.C.R., H.S.M.A. and M.J.M.B. designed the study. W.C.R., J.W.T.D., A.G.M.B., J.W.D.Z., J.A.J.W.K., P.D.L., S.F.T.T, B.J.M.V., A.J.L.W. and H.S.M.A. collected the data. W.C.R., J.W.T.D., G.C. and M.J.M.B. analysed and interpreted the data. W.C.R., J.W.T.D. and M.J.M.B. prepared the manuscript, which all authors reviewed and approved for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.07.014.

References

[1] Surveillance Atlas of Infectious Diseases. European centre for disease prevention and control. n.d. https://atlas.ecdc.europa.eu/public/index.aspx. [Accessed 6 May 2020].
[2] Voets GM, Platteel TN, Fluit AC, Scharringa J, Schapendonk CM, Cohen Stuart JWT, et al. Population distribution of beta-lactamase conferring resistance to third-generation cephalosporins in human clinical Enterobacteriaceae in The Netherlands. PLoS One 2012;7:e52102. https://doi.org/10.1371/journal.pone.0052102.
[3] Glasner C, Albiger B, Buist G, Tambić Andrasević A, Cantón R, Carmeli Y, et al. Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill 2013;18:1–7. https://doi.org/10.2807/1560-7917.ES2013.18.28.20525.
[4] Bijzonder resistente micro-organismen (BRMO). Leiden, the Netherlands: Werkgroep Infectiepreventie; 2013.
[5] Dik JWH, Sinha B, Lokate M, Lo-Ten-Foe JR, Dinkelacker AG, Postma MJ, et al. Positive impact of infection prevention on the management of nosocomial outbreaks at an academic hospital. Future Microbiol 2016;11:1249–59. https://doi.org/10.2217/fmb-2016-0030.
[6] de Kraker MEA, Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, et al. Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant Staphylococcus aureus bloodstream infections. Antimicrob Agents Chemother 2011;55:1598–605. https://doi.org/10.1128/AAC.01157-10.
[7] de Kraker MEA, Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, et al. Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to Escherichia coli resistant to third-generation cephalosporins. J Antimicrob Chemother 2011;66:398–407. https://doi.org/10.1093/jac/dkq412.
[8] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309–32. https://doi.org/10.1016/j.ajic.2008.03.002.
[9] Breakpoint tables for interpretation of MICs and zone diameters (version 8.1). The European Committee on Antimicrobial Suceptibility Testing; 2018.
[10] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2020. Vienna, Austria.
[11] Harrell Jr FE. Hmisc: Harrell miscellaneous (version 4.4-0). R package; 2020.
[12] Harrell Jr FE. Rms: regression modeling strategies (version 5.1-4). R package; 2019.
[13] van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. J Stat Softw 2011;45. https://doi.org/10.18637/jss.v045.i03.
[14] Dahl DB, Scott D, Roosen C, Magnusson A, Swinton J. xtable: export tables to LaTeX or HTML (version 1.8-4). R package; 2019.
[15] Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect 2016;22:416–22. https://doi.org/10.1016/j.cmi.2015.12.002.
[16] Kadri SS, Adjemian J, Lai YL, Spaulding AB, Ricotta E, Prevots DR, et al. Difficult-to-treat resistance in gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. Clin Infect Dis 2018;67:1803–14. https://doi.org/10.1093/cid/ciy378.
[17] Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis 2019;19:56–66. https://doi.org/10.1016/S1473-3099(18)30605-4.
[18] Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J Antimicrob Chemother 2007;60:913–20. https://doi.org/10.1093/jac/dkm318.
[19] Rottier WC, Ammerlaan HSM, Bonten MJM. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum β-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. J Antimicrob Chemother 2012;67:1311–20. https://doi.org/10.1093/jac/dks065.
[20] Stewardson AJ, Allignol A, Beyersmann J, Graves N, Schumacher M, Meyer R, et al. The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and Staphylococcus aureus in European hospitals, 2010 and 2011: a multicentre retrospective cohort study. Euro Surveill 2016;21:pii=30319. https://doi.org/10.2807/1560-7917.ES.2016.21.33.30319.
[21] Fitzpatrick JM, Biswas JS, Edgeworth JD, Islam J, Jenkins N, Judge R, et al. Gram-negative bacteraemia: a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. Clin Microbiol Infect 2016;22:244–51. https://doi.org/10.1016/j.cmi.2015.10.034.
[22] Klompas M, Calandra T, Singer M. Antibiotics for sepsis: finding the equilibrium. JAMA 2018;320:1433–4. https://doi.org/10.1001/jama.2018.12179.
[23] Ammerlaan HSM, Harbarth S, Buiting AGM, Crook DW, Fitzpatrick F, Hanberger H, et al. Secular trends in nosocomial bloodstream infections: antibiotic-resistant bacteria increase the total burden of infection. Clin Infect Dis 2013;56:798–805. https://doi.org/10.1093/cid/cis1006.
[24] de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H. The changing epidemiology of bacteraemias in Europe: trends from the European antimicrobial resistance surveillance system. Clin Microbiol Infect 2013;19:860–8. https://doi.org/10.1111/1469-0691.12028.
[25] Klein Klouwenberg PMC, Ong DSY, Bos LDJ, de Beer FM, van Hooijdonk RTM, Huson MA, et al. Interobserver agreement of Centers for Disease Control and Prevention criteria for classifying infections in critically ill patients. Crit Care Med 2013;41:2373–8. https://doi.org/10.1097/CCM.0b013e3182923712.

Please cite this article as: Rottier WC et al., Attributable mortality of antibiotic resistance in gram-negative infections in the Netherlands: a parallel matched cohort study, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2020.07.014