Journal of Infection 81 (2020) 895–901

Contents lists available at ScienceDirect

Journal of Infection

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

The burden of bacteremic and non-bacteremic Gram-negative infections: A prospective multicenter cohort study in a low-resistance country

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ARTICLE INFO

Article history:
Accepted 1 October 2020
Available online 6 October 2020

Keywords:
Burden
Gram-negative infection
Bloodstream infection
Antibiotic resistance
Mortality

SUMMARY

Objectives There is a global increase in infections caused by Gram-negative bacteria. The majority of research is on bacteremic Gram-negative infections (GNI), leaving a knowledge gap on the burden of non-bacteremic GNI. Our aim is to describe characteristics and determine the burden of bacteremic and non-bacteremic GNI in hospitalized patients in the Netherlands.

Methods We conducted a prospective cohort study of patients in eight hospitals with microbiologically confirmed GNI, between June 2013 and November 2015. In each hospital the first five adults meeting the eligibility criteria per week were enrolled. We estimated the national incidence and mortality of GNI by combining the cohort data with a national surveillance database for antimicrobial resistance.

Results 1,954 patients with GNI were included of which 758 (39%) were bloodstream infections (BSI). 243 GNI (12%) involved multi-drug resistant pathogens. 30-day mortality rate was 11.1% ($n = 217$) Estimated national incidences of non-bacteremic GNI and bacteremic GNI in hospitalized adults were 74 (95% CI 58 – 89) and 86 (95% CI 72–100) per 100,000 person years, yielding estimated annual numbers of 30-day all-cause mortality deaths of 1,528 (95% CI 1,102–1,954) for bacteremic and 982 (95% CI 688 – 1,276) for non-bacteremic GNI.

Conclusion GNI form a large mortality burden in a low-resistance country. A third of the associated mortality occurs after non-bacteremic GNI.

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https://doi.org/10.1016/j.jinf.2020.10.001

Introduction

Infections caused by Gram-negative bacteria, such as Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* species, also called Gram-negative infections (GNI), are associated with significant mortality, morbidity and health care costs[1] Such infections occur in community-dwelling subjects and may also complicate treatment in healthcare facilities as post-operative wound infections, urinary tract infections (UTI), hospital-acquired pneumonia and catheter-related bloodstream infections (CRBSI).[2] The incidence of these infections is rising, partly because of an increasing population age.[3] The global increase in antibiotic resistance increasingly hampers successful treatment.[3]

Our current understanding of the epidemiology of GNI in hospitalized patients is almost exclusively based on bloodstream infections (BSI). Additionally, studies on the burden of Gram-negative infections strongly focus on estimating the burden caused by antibiotic resistance. However, these comprise only a subset of all GNI in hospital settings and consequently, the burden arising from non-bacteremic GNI and non-resistant GNI is unknown.

We therefore aimed to comprehensively evaluate the epidemiology of GNI, including bacteremic and non-bacteremic GNI, associated pathogens, clinical management and patient outcomes in a representative cohort of hospitalized patients in the Netherlands, and used that data to estimate the national incidence and mortality associated with GNI.

Methods

Setting and study population

We conducted a prospective cohort study in eight hospitals in the Netherlands (seven secondary care hospitals, one tertiary care hospital) between June 2013 and November 2015, the GRAND-ABC study). In each hospital, trained research nurses weekly screened consecutive clinical cultures (not taken for screening purposes) of the previous week and included the first five adult patients (>18 years) that met all of the following criteria; (1) culture results involved Enterobacterales and/or non-fermenters (*Pseudomonas, Acinetobacter and Stenotrophomonas* spp.; (2) episode constituted a new infection according to the respective CDC-criteria for infection;[4] (3) for skin and pulmonary infections, prescribed antibiotics were required to cover the cultured pathogen

Definitions

Infections were considered hospital-acquired if the sample was taken =>48 h after hospital admission and healthcare-associated if the sample was taken <48 h after hospital admission and the patient had been hospitalized ≥2 nights in the last three months, was on dialysis, received intravenous therapy (e.g. chemotherapy) within the last 30 days or resided in a nursing home or other long term care facility.[5] All other infections were considered community-acquired.

Antibiotic susceptibility was tested according to local laboratory practices using EUCAST criteria and bacteria were considered Highly Resistant Microorganisms (HRMO) according to modified Dutch guidelines (supplement A). GNI were categorized as mono- or polymicrobial. In the latter case all relevant Gram-negative bacteria from index cultures were included in the analyses of antimicrobial susceptibilities.

The source of infection was based on the CDC-criteria for infections.[4] Some infections were initially included as “secondary bloodstream infection”. We retrospectively reclassified secondary bloodstream infections to the specific source if we considered it to be unambiguously clear based on the patient records.

Comorbidities were scored according to the Charlson Comorbidity Index.[6] Patients were considered immunocompromised if they had at least one of the following: (1) Chemotherapy in the last 30 days; (2) High dose corticosteroids (≥20 mg prednisone or equivalent per day) for more than two weeks at time of GNI; (3) Neutropenia ($<0.5 \times 10^9$ cells/L); or (4) Immunosuppressive drugs in the last three months.

Sepsis severity was classified according to the SEPSIS-II-guidelines as sepsis, severe sepsis or septic shock.[7] All bacteremic GNI were classified as at least sepsis. The occurrence of each of the following source control procedures was registered by trained research nurses: abscess drainage, necrotectomy, amputation, removal of prosthesis, heart valve surgery, joint flushing, other sur-

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gical procedures, insertion/exchange of bile duct stent, removal of urinary catheter and removal of central venous catheter. Other source control procedures were included if reported as such in patient charts after adjudication by one of the investigators.

Clinical outcomes included clinical cure at discharge or 14 days after GNI onset, whichever occurred first; 30-day all-cause mortality; length of stay post infection; and ICU admission post-infection. Reasons for not reaching clinical cure at day 14 were recorded.

For estimating the national burden of GNI we used data from the "Infectious Disease Surveillance Information System for Antimicrobial Resistance" (ISIS-AR).[8] This database is maintained at the national institute for public health (RIVM) and contains all positive culture results and susceptibility data from routine diagnostics in participating medical microbiology laboratories. In 2016 it was estimated that participating laboratories covered 60–75% of the hospitalized population in the Netherlands. From the database we extracted data of isolates of Enterobacterales, *Pseudomonas* spp. and *Acinetobacter* spp. from in-hospital (including emergency department) adult patients in 2016. For our analyses we included all culture results obtained in a hospital labeled as 'diagnostic' or 'unknown', thereby excluding screening cultures (e.g. rectal swabs), from hospitalized adults.

Ethics

The Ethics Committee of the University Medical Center Utrecht exempted the study from formal review and waived the requirement of informed consent due to the observational nature of the study.

Analysis

Continuous data are described in means and standard deviation or median and inter-quartile range, as appropriate. Event rates and proportions are reported as percentage with corresponding 95% confidence intervals. As this is a descriptive study, no formal statistical tests were performed.

To determine the national incidence of GNI, we related our prospective cohort findings to the ISIS-AR database. This database contains isolate data without clinical information, which may reflect a new episode of GNI or a repeated positive culture. In our prospective cohort study, we evaluated, each week, consecutive culture results (excluding upper-respiratory tract [URT] and screening cultures) to identify the first five new episodes of GNI. We were thus able to determine from the GRAND-ABC study the number of non-URT cultures required to identify one GNI episode. As an example, out of 5 urinary cultures, two may not satisfy the CDC-criteria for infection (no symptoms), two may have been taken a day after a previous urine culture for which therapy was already started (repeated culture), and one did actually involve a new UTI with symptoms and initiation of antibiotic therapy. This means the ratio of "new episode of infection" to "isolates reported" would be 1:5, or 20%. We calculation this culture-to-infection ratio for all cultures together as well as for blood cultures only.

We applied this ratio to the ISIS-AR database to estimate the total number of GNI in the Netherlands. From this number, we calculated estimates of HRMO infections and 30-day mortality by calculating those respective proportions. To take sampling variation into account, we calculated the distributions (100,000 bootstrap samples) for isolate:infection ratio (proportion), the different proportions (for prevalence of HRMO and 30-day mortality risk estimated in the GRAND-ABC database; binomial distribution) and number of cultures in the ISIS-AR database (Poisson distribution). By multiplying these distributions and taking the 2.5% and 97.5% percentiles, we calculated the 95% confidence interval. We assumed a national coverage of the ISIS-AR data of 67.5%, taking 75% for the lower bound and 60% for the upper bound of the 95% CI to generate a conservative confidence interval (see supplement B. for a flowchart of this process). Finally, we calculated the national incidence of GNI per 100,000 person-years by extrapolating the total number of GNI to the total Dutch population (13,542,471 people older than 18 years in 2016[9]). We performed all analyses separately for total number of infections, bacteremic and non-bacteremic GNI, and different ratios were applied to each.

Results

Patients

A total of 1954 GNI episodes were included, of which 725 (37.1%) were community-acquired, 681 (34.9%) were healthcare-associated, and 548 (28.0%) were hospital-onset infections (Table 1). In all, 758 (38.8%) episodes were BSI (44.0%, 43.4% and 26.1% of community-acquired, healthcare-associated and hospital-onset, respectively). The most common infection sources were the urinary tract ($n = 1008$, 51.6%), the abdomen ($n = 378$, 19.3%) and skin and soft tissue ($n = 223$, 11.4%). Samples for microbiological cultures were most often obtained at the emergency department ($n = 816$; 42%), in 28% from surgical wards ($n = 544$), in 27% from medical wards ($n = 520$) and in 74 episodes (4%) from intensive care units.

Bacteremic GNI were, compared to non-bacteremic GNI, associated with older age (71.6 vs 67.2), community onset infections (42.1% vs 33.9%) and septic shock (11.5% vs 4%), whereas non-bacteremic GNI were associated with female sex (49.4% vs 42.7%), prior surgery (23.8% vs 13.3%) and hospital-onset infections (33.9% vs 18.9%). For further stratification per infection type, see supplement C.

Second generation cephalosporins were most often prescribed as initial antimicrobial treatment for bacteremic as well as non-bacteremic GNI (overall 31.1%), followed by third-generation cephalosporins (23.3%) (Table 2). In 173 episodes (8.8%), no antibiotics were prescribed in the first 24 h after sample obtainment. Source control was performed in 664 episodes (34.0%). For a breakdown of types of source control, see supplement D.

Pathogens

The vast majority of infections was caused by a single pathogen ($n = 1661$, 85.0%), which most frequently was *E. coli* ($n = 997$; 60%), followed by *Klebsiella pneumoniae* ($n = 144$; 8.7%) and *Pseudomonas aeruginosa* ($n = 144$; 8.7%) (Table 3). The same bacterial species were predominant in polymicrobial infections. Overall, 243 infections (12.3%) involved HRMO, according to Dutch definitions; 189 (77.8% of HRMO) Enterobacterales were resistant to 3rdgeneration cephalosporins (116 being *E. coli*); 69 (28.4%) Enterobacterales were resistant to both aminoglycosides (gentamicin and/or tobramycin) and fluoroquinolones (ciprofloxacin) (48 being *E. coli*) and 2 (0.8%) Enterobacterales were resistant to carbapenems. For the pathogen and HRMO distribution by infection source, see supplement E.

Infection outcomes

All-cause 30-day mortality was 11.1% (217 of 1954); 15.6% (118 of 758) among patients with bacteremic GNI and 8.3% (99 of 1196) among those with non-bacteremic GNI (table 1). Of all patients, 1523 (78%) were considered clinically cured within 14 days and 113 (6.7%) had died within 14 days. ICU-admission after infection onset occurred in 184 cases (9.4%). Absence of clinical cure at day 14 (ongoing infection) was associated with hospital-onset infections (40.8% hospital-onset in no cure vs 24.4% in patients that were cured), prior surgery (30.4% vs 17.4%) and skin infections (27.8% vs

Table 1
Patient and infection characteristics in all, non-bacteremic GNI and bacteremic patients with Gram-negative infections in the GRAND-ABC cohort.

n	**Overall** 1954	**Non-bacteremic GNI** 1196	**Bacteremic GNI** 758
Demographics			
Age (mean (sd))	68.87 (15.7)	67.2 (16.2)	71.6 (14.1)
Female	915 (46.8)	591 (49.2)	324 (42.7)
Comorbidity			
Charlson Comorbidity index (median, IQR)	2[1–3]	2 [0 – 3]	2[1–4]
Immunocompromised	235 (12)	133 (11.1)	104 (13.7)
Prior colonization HRMO	129 (6.6)	86 (7.2)	56 (10.4)
Surgery in 30d prior	386 (19.8)	285 (23.8)	101 (13.3)
Origin			
- Community acquired	725 (37.1)	406 (33.9)	319 (42.1)
- Healthcare associated	681 (34.9)	385 (32.2)	296 (39.1)
- Hospital onset	548 (28.0)	405 (33.9)	143 (18.9)
Ward of index culture			
- Surgical ward	544 (27.8)	439 (36.7)	105 (13.9)
- Intensive care unit	74 (3.8)	38 (3.2)	36 (4.7)
- Internal medicine	520 (26.6)	341 (28.5)	179 (23.6)
- Emergency ward	816 (41.8)	378 (31.6)	438 (57.8)
LOS prior to infection (hospital-onset-infections, IQR)	8.0 [5.0 - 15.0]	8 [5.0 – 14.0]	10 [5.0 – 19.5]
Source of infection			
Primary BSI	91 (4.7)	–	91 (12.0)
Urinary tract	1008 (51.6)	602 (50.3)	406 (53.6)
Abdominal	378 (19.3)	206 (17.2)	172 (22.7)
Respiratory	165 (8.4)	142 (11.9)	23 (3.0)
Skin and soft tissue	223 (11.4)	202 (16.9)	21 (2.8)
Bone and joint	25 (1.3)	22 (1.8)	3 (0.4)
Other	64 (3.3)	22 (1.8)	42 (5.5)
Sepsis severity			
No sepsis	460 (18.4)	460 (38.5)	–
Sepsis	1193 (61.1)	614 (51.3)	579 (76.4)
Severe sepsis	164 (8.4)	72 (6.0)	92 (12.1)
Septic shock	157 (8.0)	50 (4.2)	87 (11.5)
30 day mortality	217 (11.1)	99 (8.3)	118 (15.6)

Data are given as N (%) unless otherwise indicated.

Table 2
Antibiotic treatment and source control of Gram-negative infections in the GRAND-ABC cohort.

	Total (n (%))	Non-bacteremic GNI ($n = 1028$) (%)	Bacteremic GNI ($n = 690$) (%)
Initial antibiotic treatment			
Penicillins*	365 (21.7)	202 (20.1)	163 (24.1)
2G-cephalosporins	522 (31.1)	270 (26.9)	252 (37.2)
3G-cephalosporins	392 (23.3)	197 (19.6)	195 (28.8)
Carbapenems	64 (3.8)	33 (3.3)	31 (4.6)
Fluoroquinolones	208 (12.4)	147 (14.6)	61 (9.0)
Co-trimoxazole	60 (3.6)	40 (4.0)	20 (3.0)
No AB first day after culture	173 (8.8)	121 (11.8)	52 (7.5)
Inappropriate therapy **	525 (30.6)	386 (37.5)	139 (20.1)
Source control***	664 (34.0)	436 (36.5)	228 (30.1)

*Includes amoxicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam. **Inappropriate when antibiotic therapy one day after the index culture does not cover the Gram-negative pathogen identified, includes pts not receiving an antibiotic on that day. ***Medication data missing from one hospital. Data on source control is from all hospitals.

6.8%). See supplement F for reasons of non-cure and expansions of infections.

Burden of gram-negative infections in the Netherlands

In the prospective cohort study, 14,749 Gram-negative cultures were screened to include 1954 GNI episodes, yielding a screening-infection ratio of 7.5:1. The ratios for bacteraemic and non-bacteraemic GNI were 2.0 and 10.2 respectively. The national database contained (for 2016) 97,751 cultures yielding Gram-negative bacteria. From the screening to infection episode ratio and extrapolated to the total Dutch population assuming coverage of 67.5%, we estimated a national yearly number of 19,544 GNI episodes, of which 2459 (12.5%) were HRMO infections.Using the

Table 3
Microbiological cause of Gram-negative infections in the Grand-ABC cohort.

	Overall ($n = 1954$)	Non-bacteremic GNI	Bacteremic GNI
Escherichia coli			
HRMO	997 (51.0)		
116 (11.6)	551 (46.1)		
69 (12.5)	446 (58.8)		
47 (10.5)			
E. cloacae			
HRMO	72 (3.7)		
25 (34.7)	48 (4.0)		
21 (43.8)	24 (3.2)		
4 (16.7)			
K. pneumoniae			
HRMO	144 (7.4)		
12 (8.3)	79 (6.6)		
6 (7.6)	65 (8.6)		
6 (9.2)			
P. mirabilis			
HRMO	99 (5.1)		
3 (3.0)	80 (6.7)		
3 (3.8)	19 (2.5)		
0 (0.0)			
P. aeruginosa			
HRMO	144 (7.4)		
9 (6.2)	101 (8.4)		
6 (5.9)	43 (5.7)		
3 (7.0)			
Other species			
HRMO	205 (10.5)		
22 (10.7)	143 (12.0)		
17 (11.9%)	62 (8.2)		
5 (8.1%)			
Multiple species			
- HRMO	293 (15.0)		
56 (19.1)	194 (16.2)		
43 (22.2)	99 (13.1)		
13 (13.1)			

Table 4
Estimated number (95% confidence interval) of Gram-negative infections and mortality in the Netherlands in 2016.

	GNI	GNI incidence*	Non-bacteremic GNI	Non-bacteremic GNI incidence*	Bacteremic GNI	Bacteremic GNI incidence*
Number of GNI	19,544 (16,574 –22,514)	144 (122–166)	11,613 (9707–13,519)	86 (72 - 100)	9645 (8070 – 11,221)	71 (60 – 83)
Number of HRMO infections	2459 (1887–3031)	18 (14 – 22)	1626 (1206–2047)	12 (10 – 15)	1015 (692 – 1340)	7.5 (5.1 – 9.9)
30-day mortality	2198 (1672–2723)	16 (12 – 20)	982 (688 –1276)	7.1 (5.2 –9.4)	1528 (1102–1954)	11 (8–14)
30-day mortality HRMO infections	276 (198 –354)	2.0 (1.5 – 2.6)	137 (90 –184)	1.0 (0.7 – 1.4)	161 (102–220)	1.2 (0.75 – 1.63)

GNI: gram-negative infection; HRMO: highly resistant microorganism … * per 100,000 person years. GNI, non-bacteremic and bacteremic GNI calculated separately.

day-30 mortality observed in the prospective cohort study, the national yearly estimated number of GNI-associated deaths was 2198 (Table 4). The estimated national yearly number of deaths within 30 days of infection onset was 1528 for bacteremic and 982 for non-bacteremic GNI. Extrapolated to the total Dutch population, the estimated national incidence of GNI was 144 (95% CI: 122–166) per 100,000 person years in 2016, with comparable contribution from bacteremic and non-bacteremic GNI (71 (95% CI: 60–83) and 86 (95% CI: 72–100) per 100,000 person years, respectively).

Discussion

In this study we described the epidemiology, treatment and outcomes of adult hospitalized patients with microbiologically documented GNI and estimated that the incidence of GNI in the Netherlands is 144 per 100,000 person years. Thus, our extrapolations for 2016 imply that annually around 20,000 adults develop microbiologically confirmed GNI that is diagnosed in a hospital setting and that 2200 of them succumb within 30 days of infection onset. A third of these deaths occur in patients with non-bacteremic GNI.

Previously reported national incidences of bacteremic GNI ranged from 70 to 150 per 100,000 person years in Finland, Sweden and Canada.[10–12] Our study demonstrates that there is – in the Netherlands – a more or less similar incidence of GNI that are not associated with BSI. Although non-bacteremic GNI episodes are associated with a generally less severe presentation of disease, 30-day mortality still is 8.3% in our study. For the Netherlands we estimated that annually 2198 patients die within 30 days after GNI, which is similar to reported numbers of annual deaths due to diabetes mellitus ($n = 2891$), prostate cancer ($n = 2770$) and breast cancer (3175). There are a few important caveats with this number: first, it is a description of 30-day mortality, and since people may be admitted in a hospital for other reasons, naturally, mortality does not capture the full burden of disease, which also in-

cludes surgical procedures, prolonged duration of treatment and permanent disabilities. To fully capture the burden of GNI, disability adjusted life years (DALY's) and other outcomes next to mortality should be determined.[13,14]

We consider two important aspects related to GNI epidemiology. First, antimicrobial resistance currently receives a lot of attention from researchers and policy makers. Yet, as we demonstrate for the Netherlands, 90% of GNI are caused by antimicrobial susceptible bacteria, and these bacteria are responsible for 90% of the GNI-associated 30-day mortality. We previously estimated that the attributable mortality due to antibiotic resistance in GNI in the Netherlands, which predominantly reflects resistance to third-generation cephalosporins among Enterobacterales, is close to zero.[15] Thus, interventions to solely reduce antibiotic resistant infections and transmission (like isolation strategies) are unlikely to have a major impact on the total burden of disease caused by Gram-negative bacteria. Second, our study underlines the heterogeneity of clinical presentations of GNI, which are usually described as more specific infections, such as BSI or UTI. Our findings demonstrate that such an approach will severely underestimate the true burden of disease caused by GNI.

With such a high disease incidence, prevention of GNI should be considered a public health priority. As a considerable part of the disease burden originates from community-acquired infections, interventions targeting non-hospitalized patients are warranted. Vaccination against *E. coli* infections could be an option, and a 4-valent conjugate *E. coli* vaccine, targeting four E. coli serotypes, was well tolerated and yielded good immunological response in a phase II study.[16]

For hospital-acquired infections, selective decontamination of the digestive tract (SDD) has been associated with a 50% reduction in the incidence of ICU-acquired GNI in the Netherlands.[17] SDD (or selective oral decontamination, SOD), was standard care in the ICUs of the participating hospitals in our study, which may explain the observed lower proportion of GNI diagnosed in ICU than in other studies.[12,18] Similarly, topical antibiotic prophylaxis may prevent surgical site infections in patients who undergo elective colon surgery, which is currently being adopted in Dutch hospitals.[19,20] Whether these options are suitable elsewhere is unknown and may depend on local resistance epidemiology. Other opportunities to reduce hospital-onset GNI include restrictive and proper use of urinary and intravascular catheters, and oral care for prevention of hospital acquired pneumonia.[21]

Some study limitations need to be discussed. Due to difficulties in distinguishing screening and clinical sputum samples obtained in ICU, we did not include infections from ICU patients if the microbiological evidence of infection was limited to sputum cultures. As a result, we may have underestimated the number of Gram-negative respiratory tract infections in ICUs. Second, we calculated the overall infection, bacteremic and non-bacteremic infection estimates separately, and non-bacteremic and bacteremic GNI do not add up to the overall estimate. This is because of a higher (43%) percentage of bacteremic GNI in the national database than in the cohort study (38%), due to a higher proportion of blood culture isolates in the national database compared to the isolates in our cohort study (13.1% vs 10.3%). This may reflect a true difference in the bacteraemic to non-bacteraemic infections ratio or in culture policy between hospitals participating and not participating in GRAND-ABC. As a result, we may have underestimated the relative contribution of non-bacteraemic GNI in our extrapolation to the national level.

In conclusion, we quantified the burden of Gram-negative infections in the form of incidence and all-cause mortality in a country with low levels of antibiotic resistance. One-third of the all-cause mortality in the Netherlands occurs after a non-bacteremic infection. The most important targets for reducing the burden are the community and chronic healthcare settings, where most infections develop.

Conflicts of Interest

None reported.

Funding

This work was supported by the Netherlands organisation for Healthcare Research and Development (ZonMW, project number 205200007) and R-GNOSIS (funding from the European Community's Seventh Framework Programme FP7/2007–2013 under Grant Agreement no. 282512).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.10.001.

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