GRACE

Generating evidence on Resistant bacteria in the Aged Care Environment

GRACE Investigative Study Team

2021 Report

Flinders UNIVERSITY
SAHMRI South Australian Health & Medical Research Institute
FHMRI FLINDERS HEALTH & MEDICAL RESEARCH INSTITUTE

This report was written and prepared by Lucy Carpenter, Andrew Shoubridge and Erin Flynn. Cover page was designed by Bradley Rankin. We would like to acknowledge the GRACE investigative team who designed the study, contributed to recruitment and sample processing, and consulted on the analysis:

Prof Geraint Rogers	Lead investigator/Director of Microbiome Research, SAHMRI/Flinders University
Prof Steve Wesselingh	Executive Director, SAHMRI
Prof Maria Inacio	Director of ROSA, SAHMRI
Prof Maria Crotty	Director of Rehabilitation Medicine, Flinders Medical Centre
Prof Craig Whitehead	Director of Rehabilitation and Aged Care, SALHN
Prof David Gordon	Head of SA Pathology, SALHN
Prof David Lynn	Director of Computational & Systems Biology Program, SAHMRI/Flinders University
Dr Lito Papanicolas	Infectious Diseases Consultant, Royal Adelaide Hospital
Dr Lex Leong	Medical Scientist, SA Pathology
Dr Kerry Ivey	Biostatistician, Harvard University/SAHMRI
Dr Andrew Shoubridge	Postdoctoral Researcher, SAHMRI
Dr Steven Taylor	Postdoctoral Researcher, SAHMRI
Ms Erin Flynn	Study Coordinator, SAHMRI
Ms Lucy Carpenter	PhD Candidate, Flinders University
Ms Josephine Collins	Research Nurse, SAHMRI
Ms Furdosa Ababor	Research Assistant, SAHMRI

Development of the Generating evidence on Resistant Bacteria in the Aged Care Environment (GRACE) 2021 Report was supported by a Medical Research Future Fund (MRFF) grant.

Generating evidence on Resistant Bacteria in the Aged Care Environment (GRACE) 2021 Report

ISBN: 978-0-6452918-1-0

For enquiries please contact:
Ms Erin Flynn
GRACE Study Coordinator
Microbiome & Host Health Programme
SAHMRI

E: erin.flynn@sahmri.com
W: www.sahmri.com

Suggested Citation
Carpenter, L., Shoubridge, A., Flynn, E., Taylor, S. Ivey, K., Rogers, G. on behalf of the GRACE Investigative Study Team, 2021, *Generating evidence on Resistant Bacteria in the Aged Care Environment (GRACE) 2021 Report*. SAHMRI, Adelaide.

Table of Contents

Abbreviations

ACAP	Aged Care Assessment Program
ACFI	Aged Care Funding Instrument
ADL	Activities of Daily Living
AMR	Antimicrobial Resistance
ARG	Antibiotic Resistance Genes
CARD	The Comprehensive Antibiotic Resistance Database
CHC	Complex Health Care
DHS	Department of Human Services
HEP	High Energy High Protein (Diet)
IPC	Infection Prevention and Control
MBS	Medicare Benefits Schedule
MDRO	Multi-Drug Resistant Organism
OP	Oropharyngeal
PAS-CIS	Psychogeriatric Assessment Scale – Cognitive Impairment Scale
PBS	Pharmaceutical Benefits Scheme
PEG	Percutaneous Endoscopic Gastrostomy
RACF	Residential Aged-Care Facility
RPKM	Reads Per Kilobase of transcript, per Million mapped reads

Introduction

In keeping with trends globally, Australia is experiencing significant ageing of its population.[1] By 2031, 21% of Australians will be over 65 years of age.[2] Of these, 6% are expected to live in residential aged care facilities (RACFs), rising to 30% for individuals over 85 years.[2, 3]

Residential aged care is recognised globally as a critical setting for monitoring antibiotic use and antimicrobial-resistant bacteria (AMR). High antibiotic prescribing rates,[4] individual susceptibility to infections, and high care needs likely provide an ideal environment for AMR transmission between residents and dissemination into the wider community. Despite this, the prevalence of AMR in asymptomatic individuals and dispersal of these within the RACF environment, is largely uncharacterised. Limiting the development of effective measures to prevent the spread and impact of AMR in residential aged care.[5, 6]

The Generating evidence on Resistant bacteria in the Aged Care Environment (GRACE) study aimed to address five questions that are fundamental to developing strategies to reduce AMR carriage in RACF residents:

1) What factors determine the types and levels of AMR carried by RACF residents?
2) To what extent is there evidence of AMR transmission between RACF residents?
3) Is interaction with the RACF built environment likely to facilitate AMR transmission?
4) Do hospital visits for acute care significantly influence types and levels of AMR carriage?
5) To what extent do ageing-associated changes in gut microbiology influence AMR carriage?

GRACE was a cross-sectional study supported by a Medical Research Future Fund (MRFF) grant (GNT1152268) involving five aged care facilities in metropolitan Adelaide, Australia. Participants were invited to provide stool and oropharyngeal samples for metagenomic analysis to determine microbiome and resistome characteristics. Environmental samples were collected from sites within each facility to determine the role of the environment in AMR transmission. The study also accessed Pharmaceutical Benefits Scheme and Medicare Benefits Schedule data for consenting participants. Data on clinical care, facility management practices, including cleaning, provision of care, and staffing, were obtained directly from RACF providers.

This report provides an overview of participant demographics, health status and comorbidities, medication and health system utilisation, facility characteristics, and a preliminary analysis of faecal and oropharyngeal microbiota composition and resistome. Analysis of environmental samples collected from participating sites is not included. Study data are presented prior to integrative analysis to address the five study aims.

Recruitment and Sample Collection

Three residential aged care providers and five facilities participated in the GRACE study. Within these facilities, 403 residents met the study eligibility criteria and 344 were approached to participate. A total of 279 residents consented to the study, a final recruitment rate of 75% (excluding Site 1 as this data were not available) (Fig. 1, Fig. 2A). Eleven couples across four sites enrolled in the study.

Of those who consented, 111 (39.8%) provided self-consent and 168 (60.2%) provided third-party consent. Stool samples were collected from 213 participants, and 204 were of sufficient quality for sequencing. OP swabs were collected from 252 participants, of which 237 were of appropriate quality for sequencing. Primary reasons for being unable to collect a stool sample included cognitive impairment but self-toileting (n=23), staff unable to collect (n=20), refusal (n=15) and cessation of the study due to COVID-19 (n=8). Reasons for being unable to collect an OP swab included cessation of the study due to COVID-19 (n=6) and refusal (physical and verbal) (n=16) Both sample types were collected from 194 participants.

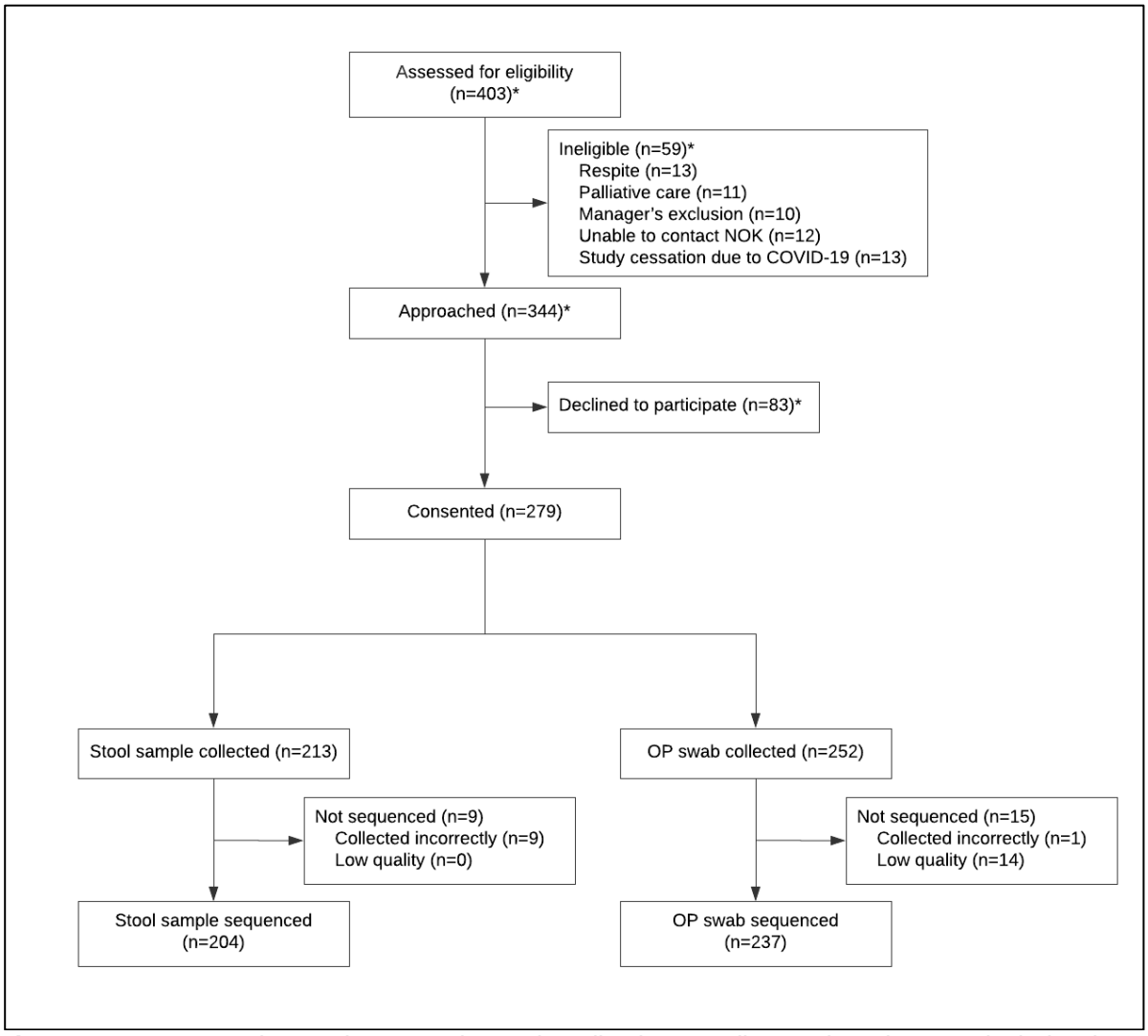


Figure 1. *GRACE study recruitment and sample collection. * Indicates data does not include Site 1. n=194 participants gave both sample types.*

Two-hundred and seventy-three residents provided consent to access Medicare benefits schedule (MBS) and pharmaceutical benefits scheme (PBS) data via the Department of Human Services (DHS) (Appendix A/B). DHS data was not accessible for those who provided incorrect supporting documentation (n=14) or completed the consent form incorrectly (n=11 for PBS and n=8 MBS). Finally, DHS could not provide PBS history for 20 participants and MBS history for 8 participants for reasons unknown to the study team. In total, 228 residents had accessible PBS data and 243 had accessible MBS data for analysis (Appendix B).

Site 3 was the largest site with 148 consenting residents, followed by site 5 (n=47) and site 4 (n=46) (Fig. 2B). Site 2 was the smallest site with 27 beds and 18 consenting participants. Site 1 was a pilot site with 20 residents recruited out of 110 occupied beds at the time of recruitment. Data on eligibility and consent was not adequately recorded. Study recruitment ceased in March 2020 due to the COVID-19 pandemic.

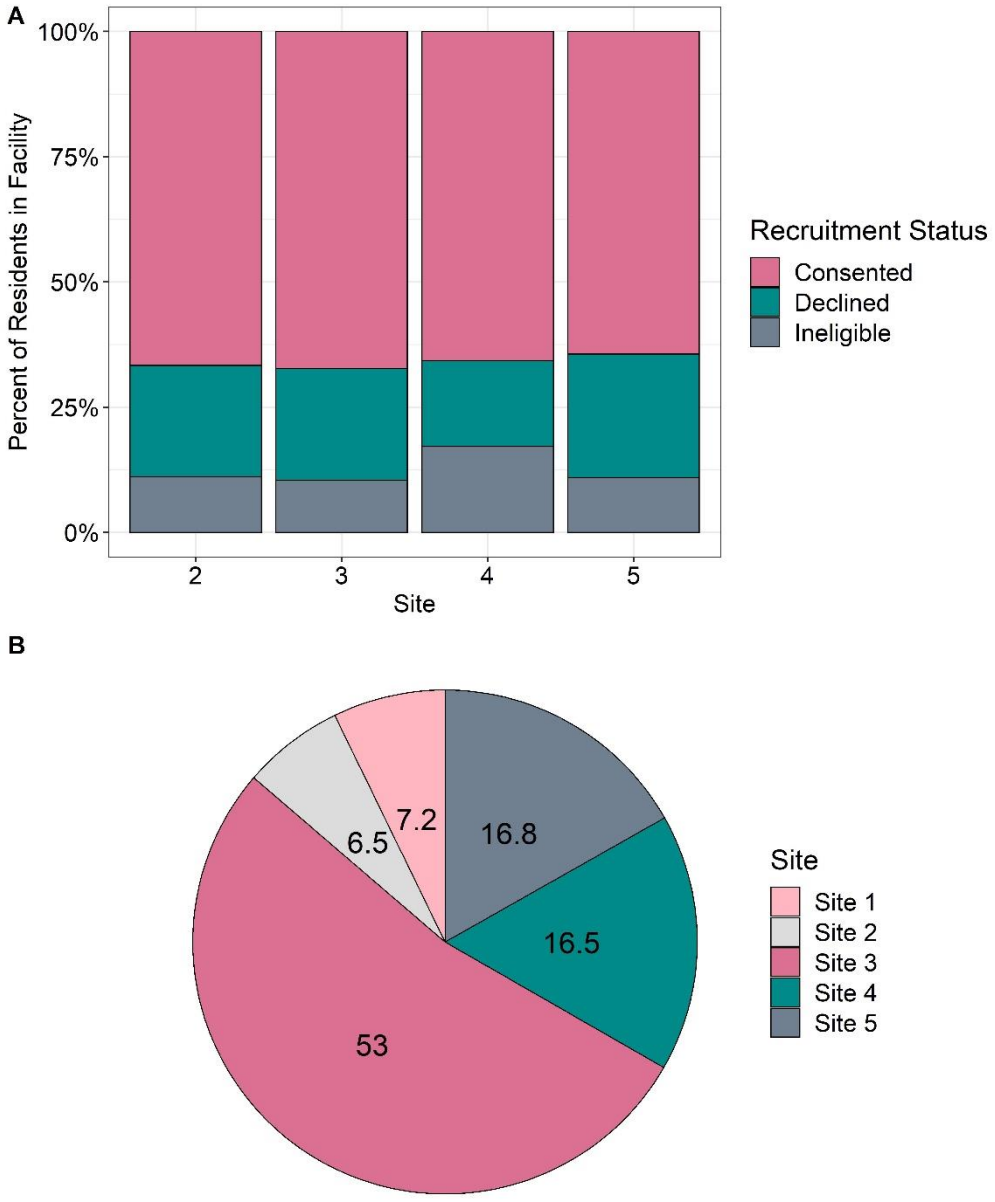


Figure 2. *Recruitment per site's total number of occupied beds for the GRACE study. Site 1 is not shown as this data was not collected (A). Percentage of total number of participants from each site (n=279) (B).*

Chapter 1: Facility Characteristics

1.1 Facility demographics

All three aged care providers were not-for-profit organisations and sites were located in South Australian metropolitan areas. Site 1 was run by provider A, sites 2 and 3 were from provider B, and sites 4 and 5 were managed by provider C.

Facility data was collected from all sites except for site 5 due to sudden cessation of the study from the COVID-19 pandemic, and therefore has limited variables available to report (Table 1). Site 4 was the oldest site, opened in 1963, and site 2 was the youngest site, opened in 2017. This was reflected in residents' average length of stay, with site 2, having an average of 283 days and site 4 an average of 949 days. Of the five sites, three had a memory support unit (sites 1,3 and 5). All sites had shared or public toilets, with only site 4 reporting shared bath facilities. Cooking and laundry of personal clothing were done in-house for all sites, while laundry for linen was outsourced.

Table 1. *Characteristics of facilities that participated in the GRACE study.*

	Site 1	Site 2	Site 3	Site 4	Site 5
Provider	A	B	B	C	C
Year opened	2012	2017	2009	1963	*NA*
Total beds (No.)	110	27	225	70	87
Occupied beds at time of recruitment (No.)	*NA*	27	220	70	86
Single rooms (No.)	110	27	225	70	*NA*
Shared rooms (No.)	0	0	0	0	*NA*
Average length of stay (days)					
Past 12 months	*NA*	283	624	949	*NA*
Past 3 years	*NA*	357	662	1022	*NA*
Memory support unit (Y/N)	Yes	No	Yes	No	Yes
Shared bath facilities (Y/N)	No	No	No	Yes	*NA*
Shared/public toilets (Y/N)	Yes	Yes	Yes	Yes	*NA*
Animals/pets onsite (Y/N)	Yes	Yes	Yes	No	*NA*
Food cooked fresh onsite (Y/N)	Yes	Yes	Yes	Yes	*NA*
Internal laundry (Y/N)					
Personal laundry	Yes	Yes	Yes	Yes	*NA*
Linen	No	No	No	No	*NA*

NA = not available

1.2 Facility healthcare management and infection control

Site 1 was the only site to report not having a hospital avoidance policy in place, and site 4 was the only site to report having an antimicrobial stewardship policy in place at the time of recruitment. Two out of the four sites had a polypharmacy review policy (sites 1 and 3), and all participated in the aged-care national antimicrobial prescribing survey (acNAPS).

No facility had hand sanitiser available inside resident rooms, and only site 1 reported having hand sanitiser available directly outside of resident rooms. Handwashing stations outside resident rooms ranged between 0.1 and 0.22 stations per room. All sites provided staff with formal hand hygiene training but only sites 2 and 3 reported having a dedicated infection prevention and control (IPC) nurse. As a result of COVID-19, all facilities in Australia must now appoint a nurse as the IPC site lead.

All sites except Site 4 reported having an infectious outbreak in the past 12 months, with sites 1 and 2 reporting a respiratory virus outbreak and sites 1 and 3 reporting a gastrointestinal virus outbreak.

1.3 Facility cleaning

Room cleans were performed weekly in all sites and high touch-point cleans performed daily in all sites except site 1, which reported daily to weekly touch-point cleans. All sites reported cleaning light switches, door handles, toilet seats, toilet flushes, and resident overways (Fig. 3). Bed remotes and call bells were cleaned only in sites 1 and 2, and TV remotes were cleaned in all sites but site 4. Sink taps were cleaned in all sites but site 4, and walking frames were cleaned only in sites 1 and 3.

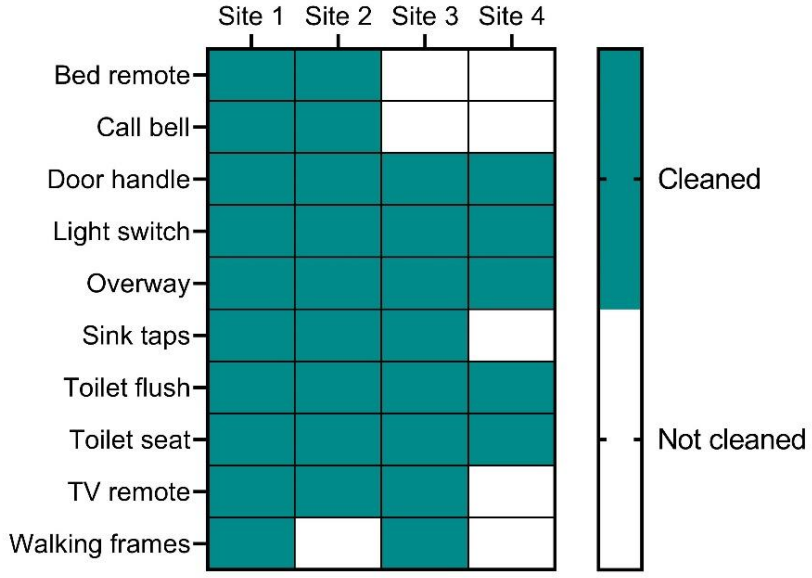


Figure 3. *Binary heatmap of surfaces reported as cleaned during high touch-point cleaning in each facility. Site 5 is not shown as this data was not available.*

Chapter 2: Participant Characteristics

2.1 Demographics

Participants of the GRACE study were a median of 88.6 years old (IQR: 11.3, Fig. 4A) with participants at site 4 the youngest (med=85.4, IQR=16.3) and participants at site 2 the oldest (med=90.3, IQR=6.8) (Fig. 4B).

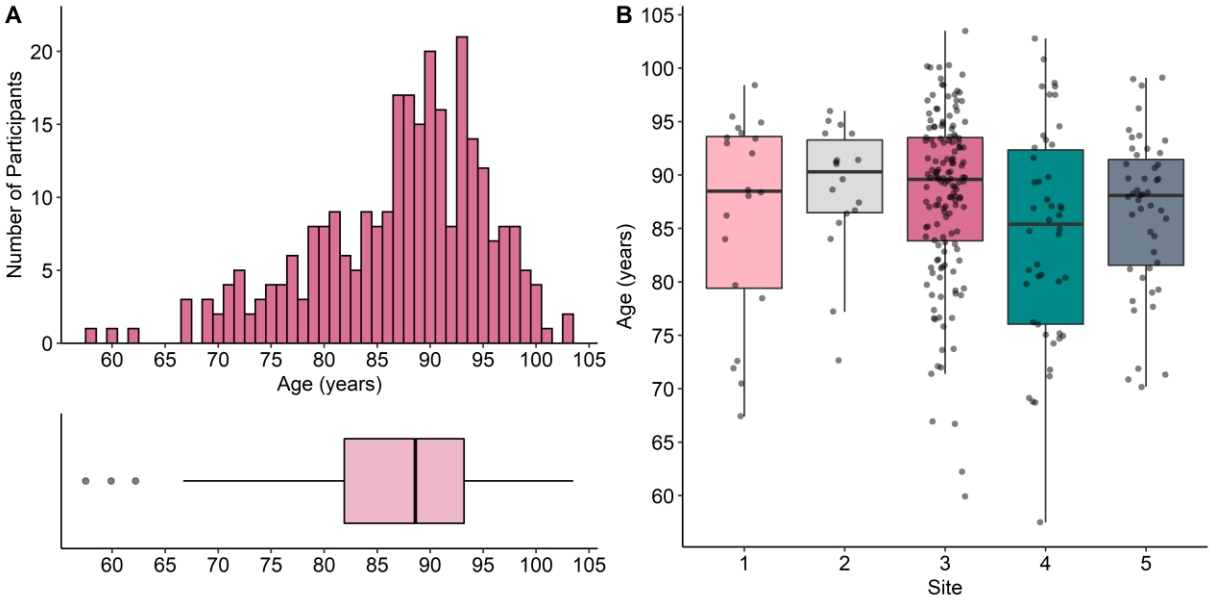


Figure 4. *Distribution of age and boxplot showing median, IQR and range of age within the entire GRACE population (A) and per site (B), with each dot representing an individual.*

Of the entire study cohort, 71.7% were female and 28.3% were male (Table 2). Ratio of males to females was consistent across enrolled participants in each site (Fig. 5A). Females enrolled in the GRACE study were generally older than males (females: med=89.4 years; males: med=85.8 years; Fig. 5B).

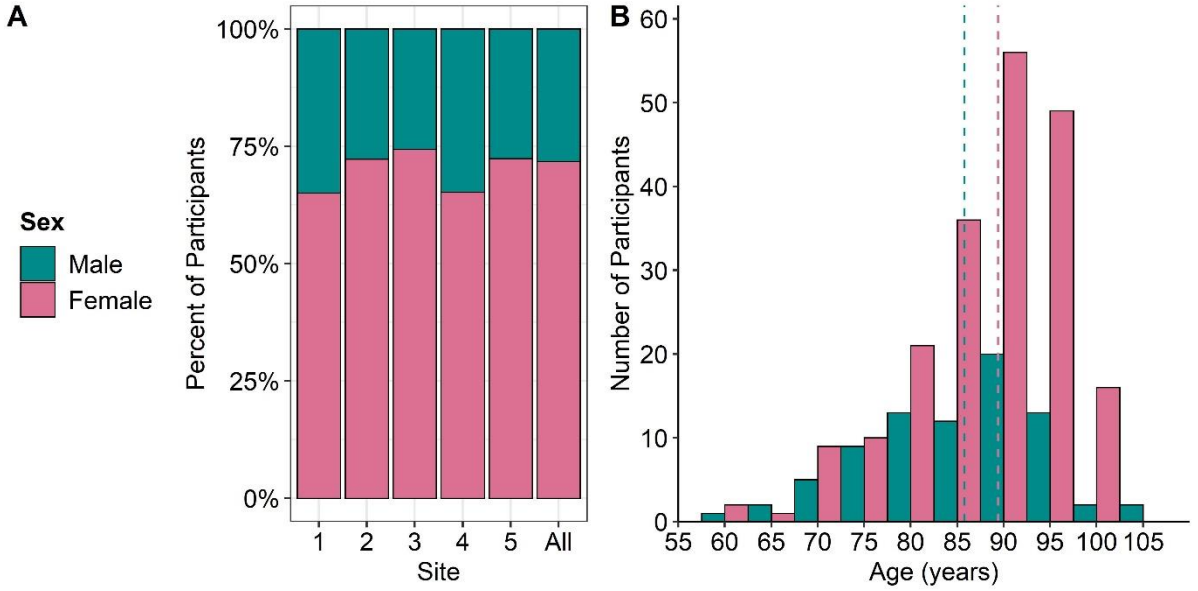


Figure 5. *Proportion of enrolled males and females per site (A) and age distribution of each sex (B). Dashed line represents the median age in years for each sex.*

Table 2. *GRACE participant characteristics.*

	Site 1 N (%)	Site 2 N (%)	Site 3 N (%)	Site 4 N (%)	Site 5 N (%)	Total N (%)
Total	20 (7.2)	18 (6.5)	148 (53.0)	46 (16.5)	47 (16.8)	279 (100)
Sex						
Female	13 (65.0)	13 (72.2)	110 (74.3)	30 (65.2)	34 (72.3)	200 (71.7)
Male	7 (35.0)	5 (27.8)	38 (25.7)	16 (34.8)	13 (27.7)	79 (28.3)
Age (years)						
< 70	1 (5.0)	0 (0)	4 (2.7)	4 (8.7)	0 (0)	9 (3.2)
70 – 74	3 (15.0)	1 (5.6)	5 (3.4)	5 (10.9)	4 (8.5)	18 (6.5)
75 - 79	2 (10.0)	1 (5.6)	13 (8.8)	5 (10.9)	5 (10.6)	26 (9.3)
80 – 84	1 (5.0)	1 (5.6)	20 (13.5)	8 (17.4)	7 (14.9)	37 (13.2)
85 – 89	4 (20.0)	6 (33.3)	38 (25.7)	11 (23.9)	16 (34.0)	75 (26.9)
90 – 94	7 (35.0)	7 (38.9)	42 (28.4)	6 (13.0)	11 (23.4)	73 (26.2)
95 – 99	2 (10.0)	2 (11.1)	21 (14.2)	5 (10.9)	4 (8.5)	34 (12.2)
> 100	0 (0)	0 (0)	5 (3.4)	2 (4.3)	0 (0)	7 (2.5)
Memory support room						
Yes	6 (30.0)	0 (0)	29 (19.6)	0 (0)	1 (2.1)	36 (12.9)
No	14 (70.0)	18 (100)	119 (80.4)	46 (100)	46 (97.9)	243 (87.1)
Shared room						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	6 (12.8)	6 (2.2)
No	20 (100)	18 (100)	148 (100)	46 (100)	41 (87.2)	273 (97.8)
Time spent in care (days)						
< 50	1 (5.0)	1 (5.6)	14 (9.5)	2 (4.3)	3 (6.4)	21 (7.5)
50 – 99	1 (5.0)	0 (0)	11 (7.4)	3 (6.5)	2 (4.3)	17 (6.1)
100 – 499	6 (30.0)	10 (55.6)	18 (12.2)	18 (39.1)	13 (27.7)	65 (23.3)
500 – 999	2 (10.0)	7 (38.9)	55 (37.2)	6 (13.0)	14 (29.8)	84 (30.1)
1000 – 1499	3 (15.0)	0 (0)	24 (16.2)	4 (8.7)	8 (17.0)	39 (14.0)
1500 – 1999	6 (30.0)	0 (0)	11 (7.4)	4 (8.7)	1 (2.1)	22 (7.9)
2000 – 2499	1 (5.0)	0 (0)	6 (4.1)	4 (8.7)	4 (8.5)	15 (5.8)
2500 – 2999	0 (0)	0 (0)	5 (3.4)	1 (2.2)	1 (2.1)	7 (2.5)
> 3000	0 (0)	0 (0)	4 (2.7)	4 (8.7)	1 (2.1)	9 (3.2)
Urinary catheter *in situ*						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	20 (100)	18 (100)	148 (100)	46 (100)	47 (100)	279 (100)
Urostomy						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

	Site 1 N (%)	Site 2 N (%)	Site 3 N (%)	Site 4 N (%)	Site 5 N (%)	Total N (%)
No	20 (100)	18 (100)	148 (100)	46 (100)	47 (100)	279 (100)
Vascular catheter *in situ*						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	20 (100)	18 (100)	148 (100)	46 (100)	47 (100)	279 (100)
Tracheostomy						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	20 (100)	18 (100)	148 (100)	46 (100)	47 (100)	279 (100)
Colostomy/Ileostomy						
Yes	0 (0)	0 (0)	7 (4.7)	0 (0)	0 (0)	7 (2.5)
No	20 (100)	18 (100)	141 (95.3)	46 (100)	47 (100)	272 (97.5)
Receiving wound care^			**n=147**	**n=45**		**n=277**
None	15 (75.0)	16 (88.9)	112 (75.7)	26 (56.5)	36 (76.6)	206 (73.8)
Multiple	0 (0)	0 (0)	5 (3.4)	3 (6.5)	1 (2.1)	9 (3.2)
Skin tear	0 (0)	0 (0)	7 (4.7)	6 (13.0)	2 (4.3)	15 (5.4)
Pressure ulcer (grade 1-2)	0 (0)	1(5.6)	11 (7.4)	4 (8.7)	2 (4.3)	17 (6.1)
Pressure ulcer (grade 3-4)	0 (0)	0 (0)	0 (0)	2 (4.3)	0 (0)	2 (0.7)
Leg ulcer	2 (10.0)	1(5.6)	1 (0.7)	0 (0)	1 (2.1)	5 (1.8)
Burn/scald	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Abrasion/graze	1 (5.0)	0 (0)	3 (2.0)	0 (0)	2 (4.3)	6 (2.2)
Surgical	0 (0)	0 (0)	2 (1.4)	0 (0)	0 (0)	2 (0.7)
Lesion	0 (0)	0 (0)	0 (0)	2 (4.3)	0 (0)	2 (0.7)
Unspecified	2 (10.0)	0 (0)	6 (4.1)	2 (4.3)	3 (6.4)	13 (4.7)
Known carriage of MDRO						
Yes	4 (20.0)	2 (11.1)	8 (5.4)	2 (4.3)	0 (0)	16 (5.7)
No	16 (80.0)	16 (88.9)	140 (94.6)	44 (95.7)	47 (100)	263 (94.3)
Diet type^	**n=19**					**n=278**
Normal	17 (85.0)	18 (100)	139 (93.9)	44 (95.7)	44 (93.6)	262 (93.9)
Vegetarian	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	1 (0.4)
Lactose free	1 (5.0)	0 (0)	7 (4.7)	1 (2.2)	2 (4.3)	11 (3.9)
Gluten free	0 (0)	0 (0)	1 (0.7)	0 (0)	1 (2.1)	2 (0.7)
Halal (no pork)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hindu (no beef)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lactose and gluten free diet	1 (5.0)	0 (0)	0 (0)	1 (2.2)	0 (0)	2 (0.7)
Prescribed meal texture						
Regular	16 (80.0)	15 (83.3)	101 (68.2)	34 (73.9)	37 (78.7)	203 (72.8)
Finger food	1 (5.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)

	Site 1 N (%)	Site 2 N (%)	Site 3 N (%)	Site 4 N (%)	Site 5 N (%)	Total N (%)
Soft	2 (10.0)	3 (16.7)	15 (10.1)	9 (19.6)	7 (14.9)	36 (12.9)
Minced and moist	0 (0)	0 (0)	18 (12.2)	2 (4.3)	2 (4.3)	22 (7.9)
Pureed	1 (5.0)	0 (0)	14 (9.5)	1 (2.2)	1 (2.1)	17 (6.1)
Liquidised	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Prescribed liquid texture						
Normal/Thin	19 (95.0)	17 (94.4)	132 (89.2)	43 (93.5)	44 (93.6)	255 (91.4)
Slightly thick	0 (0)	0 (0)	1 (0.7)	1 (2.2)	2 (4.3)	4 (1.4)
Mildly thick	0 (0)	1 (5.6)	10 (6.8)	2 (4.3)	1 (2.1)	14 (5.0)
Moderately thick	1 (5.0)	0 (0)	3 (2.0)	0 (0)	0 (0)	4 (1.4)
Extremely thick	0 (0)	0 (0)	2 (1.4)	0 (0)	0 (0)	2 (0.7)
Prescribed nutritional supplement^	**n=12**		**n=147**			**n=270**
Standard (fortified diet)	3 (15.0)	8 (44.4)	93 (62.8)	24 (52.2)	29 (61.7)	157 (56.3)
High energy & high protein (HEP)	6 (30.0)	10 (55.6)	54 (36.5)	22 (47.8)	18 (38.3)	110 (39.4)
Oral nutrition supplement	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PEG nutrition supplement	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
HEP and oral nutritional supplements	3 (15.0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.1)

^ missing data: receiving wound care, 0.7%; diet type, 0.4%; prescribed nutritional supplement, 3.2%.

At the time of enrolment, study participants had resided in their facility for a median of 681 days (IQR=878; Fig. 6A). Participants in site 1 had the longest length of stay (med=872, IQR=1454.5), and site 2 the shortest (med=457, IQR=146; Fig. 6B). This difference is likely affected by a facility's age, with site 2 opening in 2017.

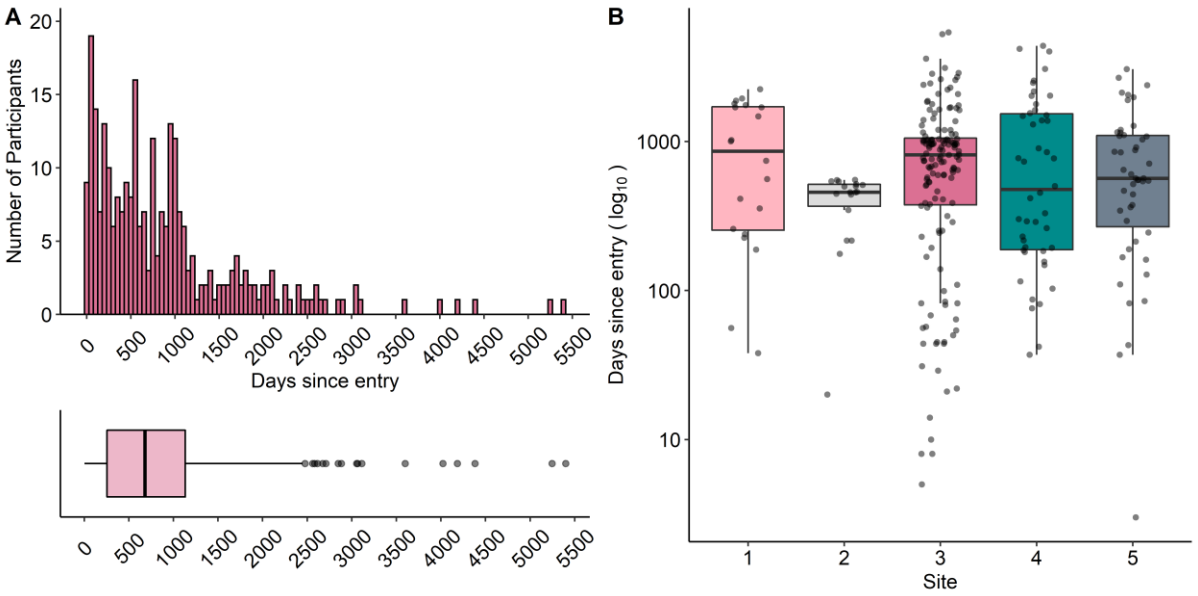


Figure 6. *Distribution and boxplot of length of time spent living at the participant's current facility for the entire GRACE cohort with the distribution overlayed (A) and per site (B).*

2.2 Diet type and supplementation

There was no difference in food preparation and supply between sites, with all reporting that food is prepared and cooked fresh on-site (Table 1). Most participants did not have any specific dietary requirements (n=262, 93.9%; Table 2/Fig. 7A), and this was consistent across sites. Of those that did, lactose-free was the most common (n=11, 3.9%). Most participants were able to consume their meals with a regular texture (n=203, 72.8%), however soft (n=36, 12.9%), minced and moist (n=22, 7.9%) and pureed (n=17, 6.1%) were also frequent (Fig. 7B). No participant had liquidised meals. Liquid texture was consistent across sites, with most participants consuming normal/thin textured liquid (n=255, 91.4%), followed by mildly thick (n=14, 5.0%; Fig. 7C). No participants were prescribed percutaneous endoscopic gastrostomy (PEG) supplementation or oral supplementation alone. Most participants receive a standard fortified diet (n=157, 56.3%), with a large proportion also on a high energy high protein (HEP) diet (n=110, 39.4%; Fig. 7D).

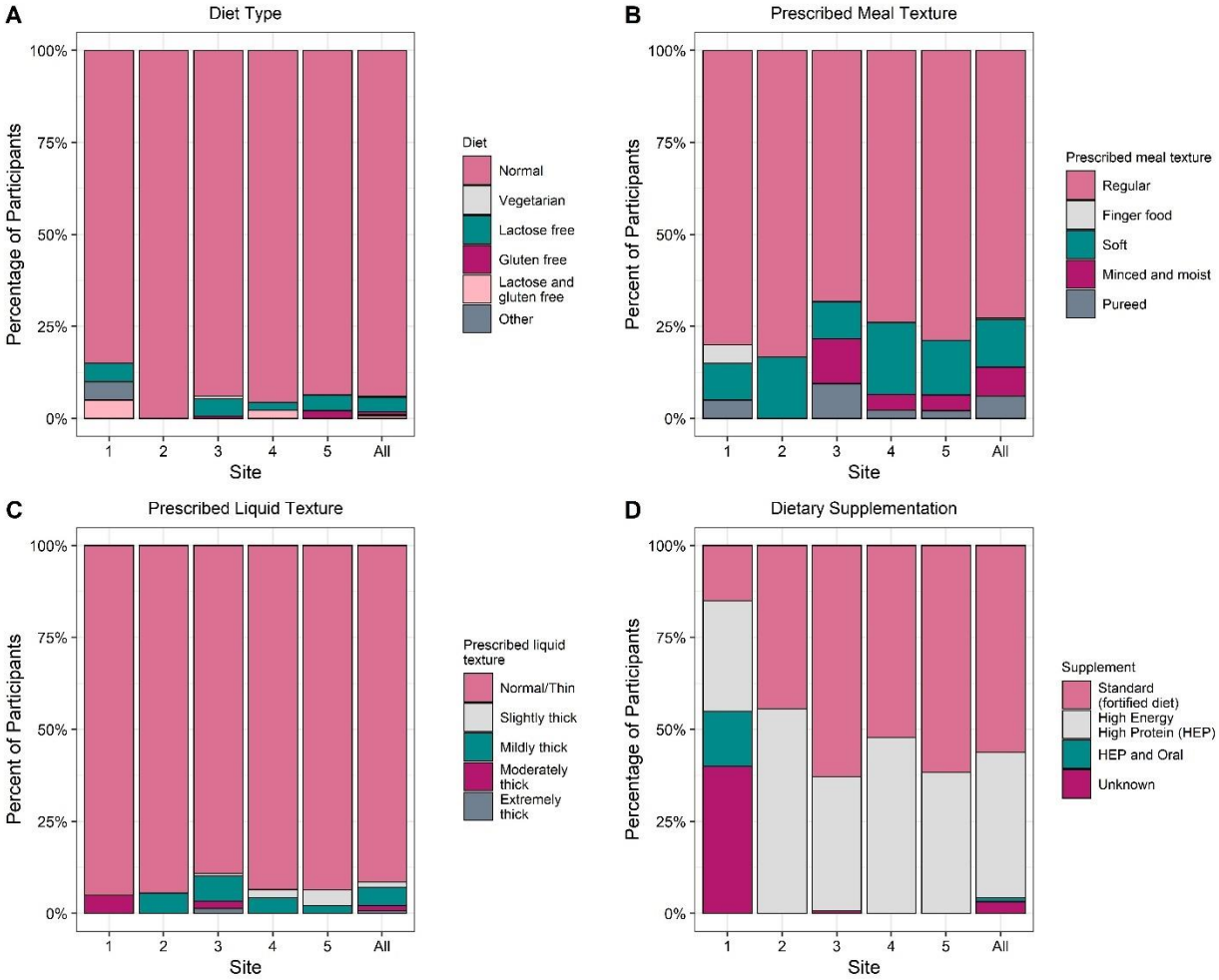


Figure 7. *Dietary requirements of GRACE study participants per site for diet type (A), meal texture (B), liquid texture (C) and dietary supplementation method (D).*

2.3 Care requirements

No study participants had a urinary catheter, vascular catheter, tracheostomy, or urostomy at the time of enrolment. Seven participants (2.5%), all from site 3, had a colostomy or ileostomy and 71 (25.6%) were receiving wound care (Table 2). Of those receiving wound care, most were for a grade 1-2 pressure ulcer (n=17, 23.9%) followed by a skin tear (n=15, 21.1%). Nine participants (12.7%) were receiving care for more than one wound. Site 4 had the highest percentage of participants receiving wound care (n=18, 39.1%) and site 2 had the lowest (n=2, 11.1%). Sixteen participants (5.7%) had carriage of an MDRO listed on their medical record. Of these, four were from site 1 (20% of enrolled participants from this site), two from site 2 (11.1%), eight from site 3 (5.4%), two from site 4 (4.3%) and none from site 5.

The Aged Care Funding Instrument (ACFI) assessment details the required levels of care for each of the three domains: Activities of Daily Living (ADL), Behaviour, and Complex Health Care (CHC) (Table 3, Appendix A). Most participants at each site were diagnosed with higher care needs for variables in the ADL category (classifications of C and D). Total ADL scores of high (C) were the most prevalent across the sites, (Fig. 8A) with a minimum 55.6% of participants at site 2, and maximum 72.3% at site 3. Collectively, the most diagnosed care level for each ADL variable for the entire cohort was C for Nutrition (77.4%), D for Mobility (67.4%), D for Personal Hygiene (88.9%), D for Toileting (74.6%), D for Continence, (83.5%), and C for Total ADL (66.0%).

Participants at each site were diagnosed with a range of care needs for Behaviour, which was reflected in the Total Behavioural category (Fig. 8B). High care needs (C) were the most common in sites 1 (65.0%), 3 (50.0%) and 5 (46.8%). Most frequently, participants at site 2 had moderate (B) care needs (55.6%). Site 4 was equally divided with 41.3% of participants classified as requiring moderate or high levels of behavioural care. Collectively, the most diagnosed care level for each Behavioural measure was C for Cognitive Skills (39.8%), A for Wandering (85.3%), D for Verbal (58.1%), A for Physical (49.8%), A for Depression (56.1%), and C for Total Behavioural (47.0%). The mean PAS-CIS score for the cohort was 9.6, with the lowest score at site 2 (8.1) and highest at site 1 (11.6). However, 48.8% of PAS-CIS scores for the cohort were recorded missing, likely due to cognitive impairment levels that were too high for the assessment to be done.

Within the CHC domain, participants at each site were assessed for the level of assistance they required for Medication and Complex Health Care procedures, which was reflected in the Total CHC category (Fig. 8C). A minimum of 55.0% of participants required high care at site 1, 61.1% at site 2, 65.5% at site 3, 65.2% at site 4, and 66.0% at site 5. Collectively, the most diagnosed care level for each factor of the Complex Health Care domain for the entire cohort was B for Medication (81.7%), D for Complex Health Care (63.4%), and C for Total CHC (64.5%).

Table 3. *GRACE participant ACFI assessment for each facility, and the combined cohort.*

	Site 1 N (%)				Site 2 N (%)				Site 3 N (%)			
Total Participants^	20 (7.2)				18 (6.5)				148 (53.0)			
Activities of Daily Living (ADL) Domain	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Nutrition	2 (10.0)	2 (10.0)	11 (55.0)	4 (20.0)	0 (0)	2 (11.1)	15 (83.3)	1 (5.6)	1 (0.7)	6 (4.1)	121 (81.8)	19 (12.8)
Mobility	0 (0)	1 (5.0)	8 (40.0)	10 (50.0)	0 (0)	0 (0)	5 (27.8)	13 (72.2)	2 (1.4)	3 (2.0)	33 (22.3)	109 (73.7)
Personal Hygiene	0 (0)	3 (15.0)	1 (5.0)	15 (75.0)	0 (0)	0 (0)	0 (0)	18 (100)	0 (0)	3 (2.0)	4 (2.7)	140 (94.6)
Toileting	0 (0)	3 (15.0)	5 (25.0)	11 (55.0)	0 (0)	0 (0.)	3 (16.7)	15 (83.3)	0 (0)	5 (3.4)	20 (13.5)	122 (82.4)
Continence	3 (15.0)	2 (10.0)	0 (0.0)	14 (70.0)	2 (11.1)	3 (16.7)	1 (5.6)	12 (66.7)	10 (6.8)	2 (1.4)	4 (2.7)	131 (88.5)
Total ADL	4 (20.0)	4 (20.0)	12 (60.0)	-	1 (5.6)	7 (38.9)	10 (55.6)	-	7 (4.7)	33 (22.3)	107 (72.3)	-
Behaviour Domain	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Cognitive Skills	0 (0)	7 (35.0)	4 (20.0)	8 (40.0)	0 (0)	10 (55.6)	6 (33.3)	2 (11.1)	3 (2.0)	28 (18.9)	61 (41.2)	55 (37.2)
Wandering	14 (70.0)	0 (0)	1 (5.0)	4 (20.0)	14 (77.8)	4 (22.2)	0 (0)	0 (0)	126 (85.1)	6 (4.1)	4 (2.7)	11 (7.4)
Verbal Behaviour	2 (10.0)	2 (10.0)	1 (5.0)	14 (70.0)	1 (5.6)	3 (16.7)	8 (44.4)	6 (33.3)	13 (8.8)	23 (15.5)	30 (20.3)	81 (54.7)
Physical Behaviour	7 (35.0)	1 (5.0)	2 (10.0)	9 (45.0)	14 (77.8)	1 (5.6)	1 (5.6)	2 (11.1)	62 (41.9)	19 (12.8)	34 (23.0)	32 (21.6)
Depression	10 (50.0)	5 (25.0)	1 (5.0)	3 (15.0)	2 (11.1)	11 (61.1)	4 (22.2)	1 (5.6)	83 (56.1)	30 (20.3)	17 (11.5)	17 (11.5)
Behavioural PAS CIS (mean (SD))	11.6 (5.4)				8.1 (3.6)				9.3 (4.5)			
Total Behavioural	3 (15.0)	4 (20.0)	13 (65.0)	-	5 (27.8)	10 (55.6)	3 (16.7)	-	22 (14.9)	49 (33.1)	74 (50.0)	-
Complex Health Care (CHC) Domain	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Medication*	1 (5.0)	14 (70.0)	4 (20.0)	-	1 (5.6)	15 (83.3)	2 (11.1)	-	1 (0.7)	122 (82.4)	14 (9.5)	10 (6.8)
Complex Health Care	1 (5.0)	0 (0)	8 (40.0)	10 (50.0)	1 (5.6)	1 (5.6)	2 (11.1)	14 (77.8)	0 (0)	11 (7.4)	44 (29.7)	92 (62.2)
Total CHC	2 (10.0)	7 (35.0)	11 (55.0)	-	3 (16.7)	4 (22.2)	11 (61.1)	-	9 (6.1)	41 (27.7)	97 (65.5)	-

	Site 4 N (%)				Site 5 N (%)				Total N (%)			
Total Participants	46 (16.5)				47 (16.8)				279 (100)			
Activities of Daily Living (ADL) Domain	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Nutrition	0 (0)	5 (10.9)	37 (80.4)	4 (8.7)	0 (0)	4 (8.5)	32 (68.1)	8 (17.0)	3 (1.2)	19 (6.8)	216 (77.4)	36 (12.9)
Mobility	0 (0)	0 (0.)	18 (39.1)	28 (60.9)	0 (0)	1 (2.1)	15 (31.9)	28 (59.6)	2 (0.7)	5 (1.8)	79 (28.3)	188 (67.4)
Personal hygiene	1 (2.2)	2 (4.4)	6 (13.0)	37 (80.4)	0 (0)	1 (2.1)	5 (10.6)	38 (80.9)	1 (0.4)	9 (3.2)	16 (5.7)	248 (88.9)
Toileting	1 (2.2)	2 (4.4)	14 (30.4)	29 (63.0)	0 (0)	3 (6.4)	10 (21.3)	31 (66.0)	1 (0.4)	13 (4.7)	52 (18.6)	208 (74.6)
Continence	2 (4.4)	2 (4.4)	4 (8.7)	38 (82.6)	4 (8.5)	0 (0)	1 (2.1)	38 (80.9)	21 (7.5)	9 (3.2)	10 (3.6)	233 (83.5)
Total ADL	3 (6.5)	16 (34.8)	27 (58.7)	-	3 (6.4)	14 (29.8)	28 (59.6)	-	18 (6.5)	74 (26.5)	184 (66.0)	-
Behaviour Domain	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Cognitive skills	3 (6.5)	16 (34.8)	23 (50.0)	4 (8.7)	2 (4.3)	16 (34.0)	17 (36.2)	9 (19.2)	8 (2.9)	77 (27.6)	111 (39.8)	78 (28.0)
Wandering	42 (91.3)	1 (2.2)	0 (0)	3 (6.5)	42 (89.4)	0 (0)	0 (0)	2 (4.3)	238 (85.3)	11 (3.9)	5 (1.8)	20 (7.2)
Verbal Behaviour	6 (13.0)	2 (4.4)	4 (8.7)	34 (73.9)	10 (21.3)	3 (6.4)	4 (8.5)	27 (57.5)	32 (11.5)	33 (11.8)	47 (16.9)	162 (58.1)
Physical Behaviour	32 (69.6)	4 (8.7)	2 (4.4)	8 (17.4)	24 (51.1)	4 (8.5)	4 (8.5)	12 (25.5)	139 (49.8)	29 (10.4)	43 (15.4)	63 (22.6)
Depression	7 (15.2)	20 (43.5)	12 (26.1)	7 (15.2)	5 (10.6)	15 (31.9)	15 (31.9)	9 (19.2)	107 (38.4)	81 (29.0)	49 (17.6)	37 (13.3)
Behavioural PAS CIS (mean (SD))	9.1 (4.0)				10.3 (4.3)				9.6 (4.4)			
Total Behavioural	7 (15.2)	19 (41.3)	19 (41.3)	-	12 (25.5)	10 (21.3)	22 (46.8)	-	49 (17.6)	92 (33.0)	131 (47.0)	-
Complex Health Care Domain (CHC)	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Medication*	0 (0)	40 (87.0)	5 (10.9)	1 (2.2)	1 (2.1)	37 (78.7)	6 (12.8)	0 (0)	4 (1.4)	228 (81.7)	31 (11.1)	11 (3.9)
Complex healthcare	0 (0)	3 (6.5)	13 (28.3)	30 (65.2)	0 (0)	2 (4.3)	11 (23.4)	31 (66.0)	2 (0.7)	17 (6.1)	78 (28.0)	177 (63.4)
Total CHC	1 (2.2)	15 (32.6)	30 (65.2)	-	2 (4.3)	12 (25.5)	31 (66.0)	-	17 (6.1)	79 (28.3)	180 (64.5)	-

^ Missing data: nutrition, 1.8%; mobility, 1.8%; personal hygiene, 1.8%; toileting, 1.8%; continence, 2.2%; total ADL, 1.1%; cognitive skills, 1.8%; wandering, 1.8%; verbal behaviour, 1.8%; physical behaviour, 1.8%; depression, 1.8%; behavioural PAS CIS, 48.8%; total behavioural, 2.5%; medication, 1.8%; complex health care, 1.8%; total CHC, 1.1%.

* Indicates score of D only applicable for assessment prior to 2017

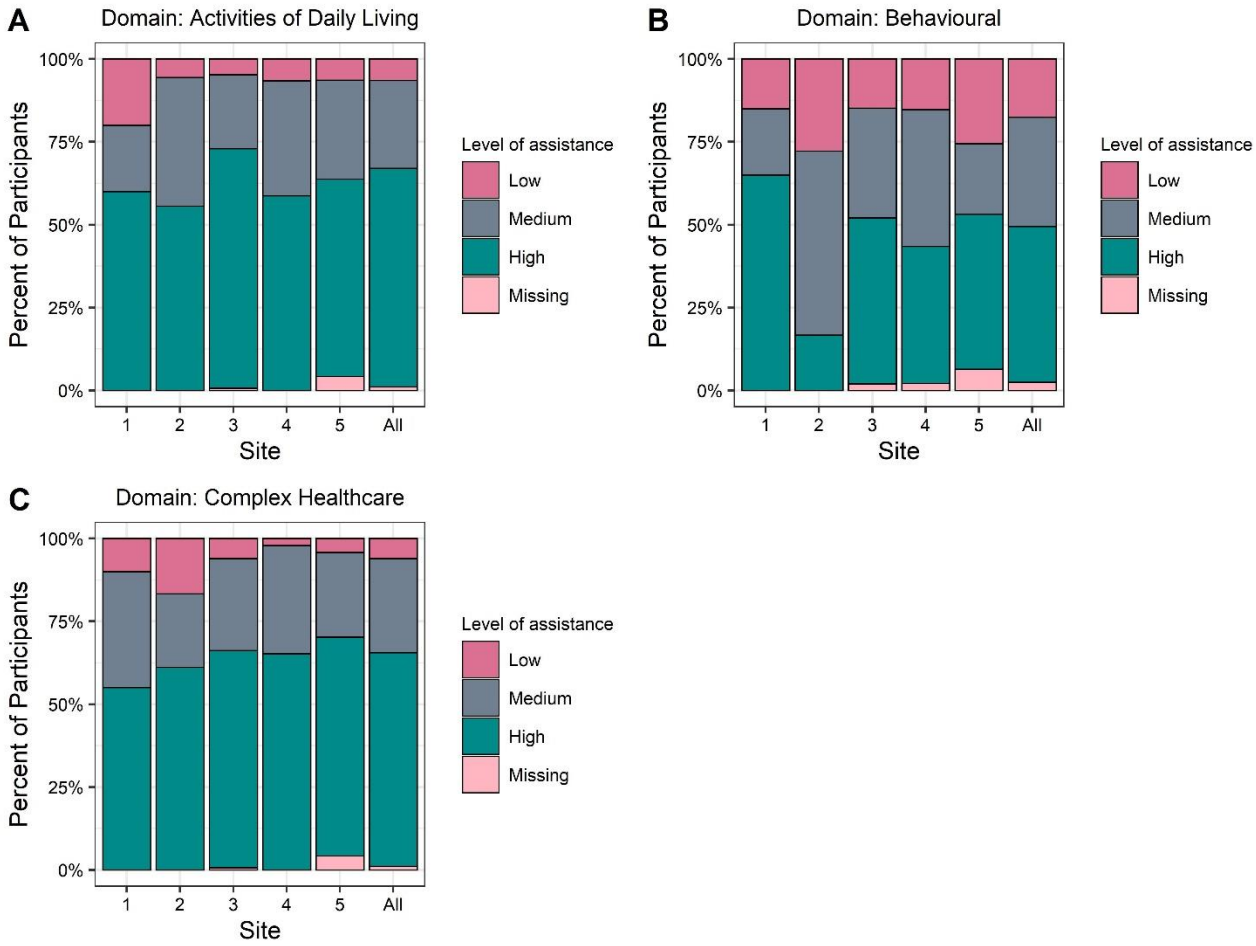


Figure 8. *ACFI care needs for participants per site and in total. Assessments for the three domains of aged care subsidised by the ACFI are summarised as total proportions of participants per site. The domains are categorised as activities of daily living (A), behaviour (B), and complex healthcare (C).*

2.4 Mental and behavioural diagnoses

Participants cognitive function and mental health was captured via ACFI Mental and Behavioural Diagnosis records (Table 4).

Table 4. *GRACE participant Aged Care Funding Instrument (ACFI) mental diagnoses for each facility and the combined cohort.*

ACFI Diagnosis	Site 1 N (%)	Site 2 N (%)	Site 3 N (%)	Site 4 N (%)	Site 5 N (%)	Total N (%)	Dementia type (%)
Dementia*	11 (55.0)	4 (22.2)	99 (66.9)	17 (37.0)	21 (44.7)	152 (54.5)	
Alzheimer's Disease	8 (40.0)	2 (11.1)	71 (48.0)	15 (32.6)	15 (31.9)	111 (39.8)	(73.0)
Vascular dementia	0 (0.00)	2 (11.1)	23 (15.5)	1 (2.2)	5 (10.6)	31 (11.1)	(20.4)
Dementia in other diseases (eg Parkinson's)	1 (5.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	(0.7)
Multiple dementias	0 (0)	0 (0)	2 (1.4)	0 (0)	1 (2.1)	3 (1.1)	(2.0)
Other dementias (eg Lewy body)	2 (10.0)	0 (0)	7 (4.7)	1 (2.2)	2 (4.3)	12 (4.3)	(7.9)
Delirium	0 (0)	0 (0)	9 (6.1)	1 (2.2)	6 (12.8)	16 (5.7)	N/A
Depression	11 (55.0)	10 (55.6)	69 (46.6)	32 (69.6)	36 (76.6)	158 (56.6)	N/A
Psychoses	0 (0)	0 (0)	7 (4.7)	1 (2.2)	3 (6.4)	11 (3.9)	N/A
Neurotic disorders	3 (15.0)	4 (22.2)	52 (35.1)	14 (30.4)	12 (25.5)	85 (30.5)	N/A
Intellectual/developmental disorders	0 (0)	0 (0)	5 (3.4)	0 (0)	0 (0)	5 (1.8)	N/A
Other disorders	1 (5.0)	0 (0)	0 (0)	0 (0)	3 (6.4)	4 (1.4)	N/A
Unknown	1 (5.0)	0 (0)	1 (0.7)	0 (0)	0 (0)	2 (0.7)	N/A
Cognitive impairment Category	PAS-CIS	PAS-CIS	PAS-CIS	PAS-CIS	PAS-CIS	PAS-CIS	N/A
Cognitive Impairment Score mean (SD)	11.56 (5.4)	8.12 (3.6)	9.32 (4.5)	9.12 (4.0)	10.33 (4.3)	9.56 (4.4)	N/A
Impairment level	**n=19**		**n=147**		**n=44**	**n=274**	
No or minimal impairment	0 (0)	0 (0)	3 (2.0)	3 (6.5)	2 (4.3)	8 (2.9)	N/A
Mild impairment	7 (35.0)	10 (55.6)	28 (18.9)	16 (34.8)	16 (34.0)	77 (27.6)	N/A
Moderate impairment	4 (20.0)	6 (33.3)	61 (41.2)	23 (50.0)	17 (36.2)	111 (39.8)	N/A
Severe impairment	8 (40.0)	2 (11.1)	55 (37.2)	4 (8.7)	9 (19.2)	78 (28.0)	N/A

^ Missing data: Impairment level, < 1.8%.
* Three participants were diagnosed with multiple types of dementia. Multiple dementia types was not a specific ACFI diagnosis; thus the total percentage exceeds 100%.

Depression was diagnosed in 56.6% of GRACE cohort participants (n=158; Fig. 9A), ranging from 46.6% in site 2 participants to 76.6% for site 5 participants. Over half of the total participants had a dementia diagnosis (n=152, 54.5%) (Fig. 9B). The highest prevalence of dementia was among participants from site 3 (66.9%) and the lowest at site 2 (22.2%). Of the different classifications of dementia, Alzheimer's disease was the most prevalent, accounting for 73.0% (n=111) of all dementia diagnoses. Vascular dementia was the next most prevalent (20.4% of dementia diagnoses, n=31 participants), followed by other dementias, such as Lewy body dementia (7.9% of dementia diagnoses, n=12 participants).

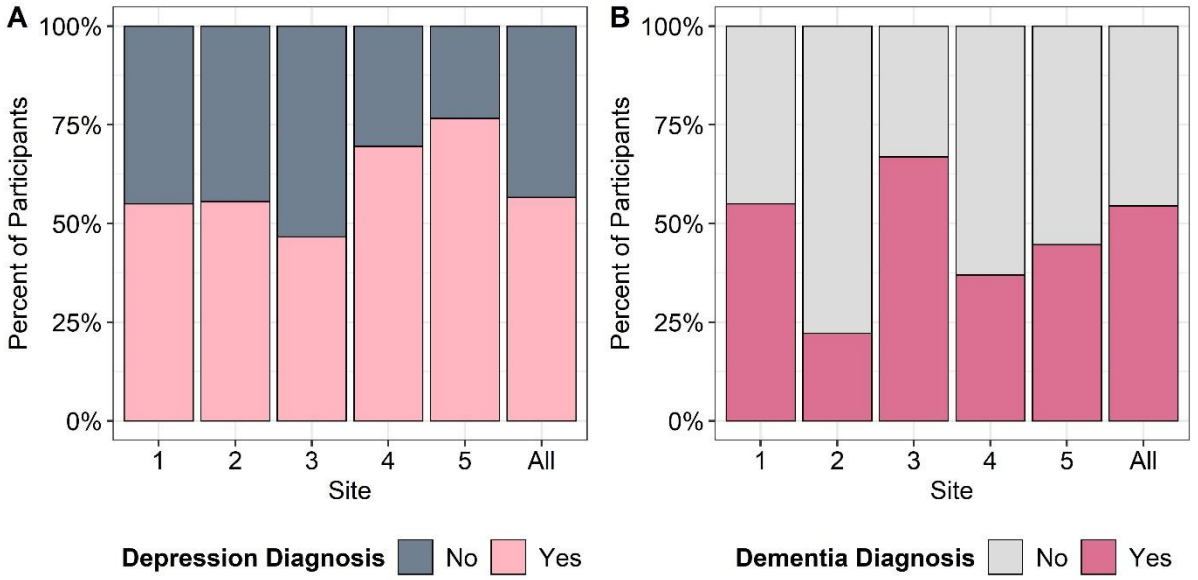


Figure 9. *Diagnosis of depression (A) and dementia (B) in the GRACE cohort.*

Of those with a known dementia diagnosis and PBS data available (n=123), 18 (14.6%) were supplied anti-dementia medication. Unusually, one participant was receiving anti-dementia medication but did not have a diagnosis of dementia reported. At least one antipsychotic medication was supplied to 18.7% (n=23) of participants diagnosed with dementia in the 12 months prior to study enrolment. At least one anxiolytic was supplied to 30.9% (n=38) of participants with a dementia diagnosis in this period, and at least one hypnotic/sedative was supplied to 8.9% (n=11) of participants.

Both depression and dementia were diagnosed in 29.4% (n=82) of participants and 18.2% were not diagnosed with either (n=51). Antipsychotics were supplied to 30 participants (13.6%) and antidepressants to 99 (43.4%) participants within 12 months before enrolment, regardless of a mental and behavioural diagnosis. Anxiolytics were supplied at least once to 57 (25.0%) participants, and hypnotics/sedatives were supplied at least once to 30 (13.6%) participants. No participants were supplied medications from all classes. Most frequently, participants were supplied both antidepressants and anxiolytics (n=13; Fig. 10A), and this was the same for participants with a dementia diagnosis (n=7; Fig. 10B).

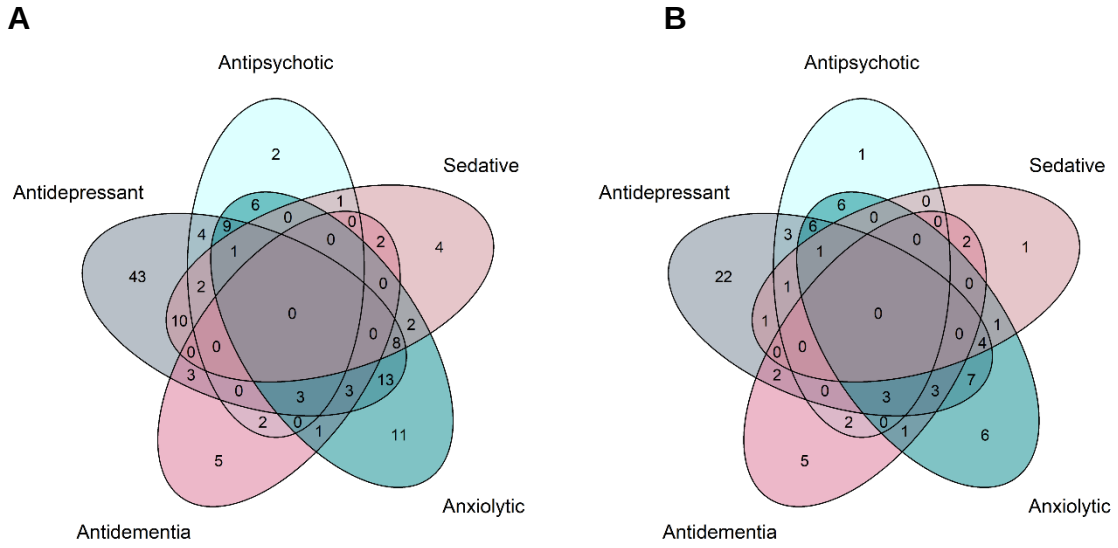


Figure 10. *Overlap between supply of antipsychotic, antidepressant, antidementia, anxiolytic and sedative medication for all GRACE participants who received at least 1 during the 12 months prior to enrolment (A) and all GRACE participants with these parameters and a dementia diagnosis (B).*

Cognitive impairment was inferred from a participant's cognitive assessment included in the ACFI. Moderate cognitive impairment was the highest assessment for 39.8% (n=111) of the entire GRACE cohort (Fig. 11). Severe impairment was classified for 28.0% (n=78) of the cohort, followed by mild (27.6%; n=77) and no or minimal impairment (2.9%; n=8). Recorded levels of cognitive impairment were most severe in sites 1, 3, and 5, and minimal to mild in sites 2 and 4.

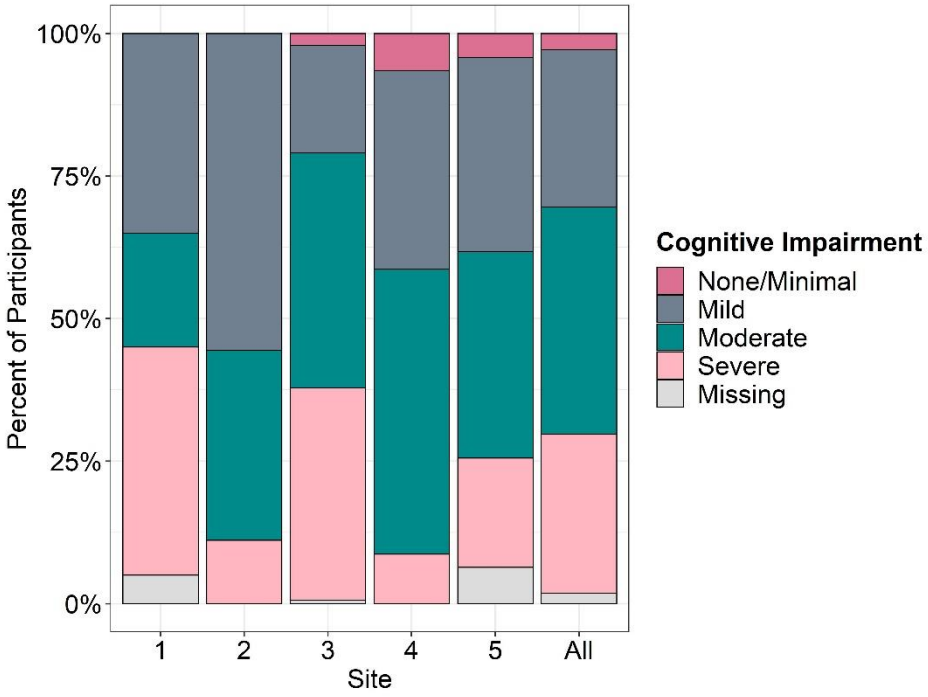


Figure 11. *Cognitive impairment levels per site and in total.*

2.5 Comorbidities

Participants of the GRACE study had their comorbidities inferred using the ACFI Aged Care Assessment Program (ACAP) diagnosis codes. Data on comorbidities was not available for participants in site 1 and was missing for one participant in site 3 (n=258). GRACE participants had a median of 11 medical conditions (excluding mental and behavioural conditions) per person (range = [2, 20]). Of all the medical conditions recorded for participants (excluding mental and behavioural diagnoses), the 5 most common included arthritis and related disorders (n=213, 82.6%), stress/urinary incontinence (n=180, 69.8%), hypertension (n=174, 67.4%), diseases of the intestine (n=103, 39.9%), and other diseases of the digestive system not elsewhere classified (n=101, 39.1%; Table 5). The full list of conditions recorded for the GRACE cohort can be found in Appendix C.

Table 5. *Most prevalent medical conditions present in total GRACE study participants as determined from their ACFI data.*

Medical Condition	Site 2 N (%)	Site 3 N (%)	Site 4 N (%)	Site 5 N (%)	Total N (%)
	n=18	n=147	n=46	n=47	n=258
Other arthritis and related disorders	13 (72.2)	128 (87.1)	33 (71.7)	39 (83.0)	213 (82.6)
Stress/urinary incontinence	9 (50.0)	106 (72.1)	31 (67.4)	37 (78.7)	180 (69.8)
Hypertension	10 (55.6)	102 (69.4)	30 (65.2)	32 (68.1)	174 (67.4)
Diseases of the intestine	10 (55.6)	65 (44.2)	11 (23.9)	17 (36.2)	103 (39.9)
Other diseases of the digestive system	5 (27.8)	55 (37.4)	20 (43.5)	21 (44.7)	101 (39.1)
Osteoporosis	7 (38.9)	54 (36.7)	12 (26.1)	15 (31.9)	88 (34.1)
High cholesterol	6 (33.3)	58 (39.5)	7 (15.2)	15 (31.9)	86 (33.3)
Other health condition not elsewhere specified	5 (27.8)	47 (32.0)	19 (41.3)	11 (23.4)	82 (31.8)
Deafness/hearing loss	6 (33.3)	43 (29.3)	6 (13.0)	14 (29.8)	69 (26.7)
Other diseases of the nervous system	3 (16.7)	33 (22.4)	11 (23.9)	20 (42.6)	67 (26.0)
Heart disease	4 (22.2)	43 (29.3)	8 (17.4)	5 (10.6)	60 (23.3)
Kidney and urinary system (bladder) disorders	5 (27.8)	29 (19.7)	11 (23.9)	14 (29.8)	59 (22.9)
Chronic lower respiratory diseases	6 (33.3)	24 (16.3)	11 (23.9)	15 (31.9)	56 (21.7)
Other heart diseases	8 (44.4)	24 (16.3)	10 (21.7)	14 (29.8)	56 (21.7)
Diabetes mellitus–type 2 (NIDDM)	2 (11.1)	26 (17.7)	10 (21.7)	14 (29.8)	52 (20.2)

Of all signs and symptoms, the median recorded per person was 3 (range = [0, 10]). The 5 most common signs and symptoms recorded for participants included falls (n=119, 46.1%), pain (n=118, 45.7%), oedema (n=107; 41.5%), bowel/faecal incontinence (n=98; 38.0%) and abnormalities of gait and mobility (n=53, 20.5%; Table 6).

Table 6. *Most prevalent symptoms and signs present in total GRACE study participants as determined from their ACFI data.*

Symptom/Sign	Site 2 N (%)	Site 3 N (%)	Site 4 N (%)	Site 5 N (%)	Total N (%)
	n=18	n=147	n=46	n=47	n=258
Falls (frequent with unknown aetiology)	12 (66.7)	73 (49.7)	15 (32.6)	19 (40.4)	119 (46.1)
Pain	5 (27.8)	65 (44.2)	16 (34.8)	32 (68.1)	118 (45.7)
Oedema (not specified)	3 (16.7)	66 (44.9)	10 (21.7)	28 (59.6)	107 (41.5)
Bowel/faecal incontinence	3 (16.7)	63 (42.9)	13 (28.3)	19 (40.4)	98 (38.0)
Abnormalities of gait and mobility	3 (16.7)	43 (29.3)	2 (4.3)	5 (10.6)	53 (20.5)

2.6 Medication usage

Participants with PBS data (n= 228) had been supplied with 311 different medications across the study period. Two-hundred and twenty-three participants had PBS data recorded in the 12 months prior to enrolment. Polypharmacy is most commonly defined as the daily usage of 5 or more medications.[7] In the context of the GRACE study, we have defined polypharmacy as the supply of 5 or more medications in the month prior to enrolment, as we are unable to determine daily usage. Of the participants with accessible PBS data, 45.6% (n=104) were taking 5 or more medications during this time and 7.0% (n=16) were taking 10 or more. The median number of medications supplied during this period was 5 and ranged from 0 to 17.

Medications used most frequently by GRACE participants included macrogol (n=82, 36.0%), furosemide (n=76, 33.3%), pantoprazole (n=69, 30.3%), and cefalexin (n=64, 28.1%). Appendix D contains a list of the top 10 most commonly used medications in GRACE participants.

2.7 Antibiotic use and microbiological pathology services

Systemic antibiotics were supplied 867 times in the 12 months prior to enrolment relative to each participant. Around 61% (n=139) of participants had been supplied at least one antibiotic in the 12 months prior to enrolment; 43% (n=98) had been supplied at least two antibiotics, and 36% (n=82) had been supplied three or more. The antibiotics supplied to the most participants in the GRACE cohort included cefalexin (n=64; 28.1%), amoxicillin and clavulanic acid (n=20; 21.9%), trimethoprim (n=44; 19.3%), amoxicillin (n=36; 15.8%), and doxycycline (n=30; 13.2%; Fig. 12). Pathology services for microbiological testing were accessed at least once by 195 residents (80.3%) in the 12 months prior to enrolment. Of all residents who accessed a pathology service for microbiology (n=195), the most common reason was for a urine examination (n=122 residents, 62.6%), followed by detection of a virus or microbial antigen or microbial nucleic acid (3 or more tests; n=50 residents, 25.6%) and microscopy and culture to detect pathogenic micro-organisms from skin or other superficial sites (n=41 residents, 21.0%).

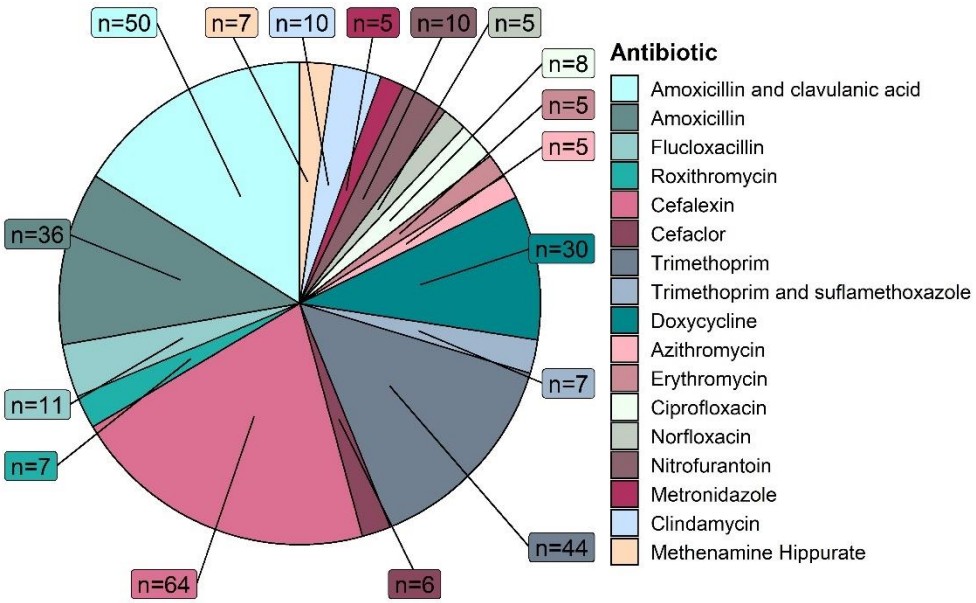


Figure 12. *Most frequently supplied antibiotics in the GRACE cohort up to 12 months prior to their enrolment. 'n=' refers to the number of residents who received this antibiotic at least once in this period.*

2.8 Access of healthcare services

Eighty-eight participants (31.5%) had a known hospital separation (either from an emergency, elective admission or other) at least once in the 12 months prior to enrolment. Eighty-four participants had the full 12-month period of data available. Of these, the median number of hospital visits in the 12 months prior to enrolment per person was 1 (range = [1, 5]). Of all the hospital events recorded (n=123 events), 92 of them were emergency visits (74.8%), 17 were elective admissions (13.8%) and 14 were unknown (11.4%). In cases where the number of days spent in hospital was recorded (n=89 events), the median number of days was 4 (range = [1, 63]). Most frequently, the indication for an emergency visit was an infection (including urinary tract infections (UTI), pneumonia and cellulitis; n=22 events; 23.9% of emergency visits), followed by falls (n=18 events; 19.6%) and heart complications (including heart failure and myocardial infarction; n=12 events; 13.0%). Antibiotics were supplied for 44.6% (n=41) of emergency events and was unknown for 15.2% (n=14) of cases, reflective of the high proportion of attendances that were for infections. Elective admissions were most commonly for surgical procedures (n=7 events; 41.2% of elective admissions), stroke rehabilitation (n=3 events; 17.6%) and falls (n=3 events; 17.6%). Antibiotics were prescribed in 6 (35.3%) cases, most likely prophylactically, and antibiotic use was unknown for 6 (35.3%) cases. Of all hospital events where antibiotics were prescribed (n=50), the most frequently given were amoxicillin with clavulanic acid (n=14 events; 28.0%) and ceftriaxone (n=10 events; 20.0%).

Of the 279 consenting participants in the GRACE study, MBS records could be accessed for 243 (87.1%) participants in the 12 months prior to enrolment (Appendix B). Items were identified in patients with at least one instance recorded, and according to the Australian Government Department of Health Medicare Benefits Schedule Book. For professional attendance items, general practitioner attendance after-hours was the most frequent, occurring for 85.2% (n=207) of participants. Diagnostic imaging services applied to 51.4% (n=125) of participants. Of these services, diagnostic radiology was the most frequent, required by 40.7% (n=99) of participants, followed by ultrasound to 29.2% (n=71) of participants. Pathology services were the most frequently recorded service to participants, applying to 93.4% (n=227) of participants. Within the listed pathological services, patient episode initiations were the most frequent, occurring in 93% (n=226) of participants. Details of services accessed by participants can be found in Appendix E.

Chapter 3: Microbiome and Resistome Characteristics

3.1 Microbiome composition of the stool

The human microbiome is defined as the community of microorganisms that inhabit the various surfaces of our bodies. Metagenomic assessment of the stool microbiome was performed for 204 (95.8%) available samples. Across all stool samples, 11 phyla were detected, consisting of 187 genera, or 586 species. A median of 101 (range = [39, 157]) species were detected per person. Of these, four were detected in 98.5% samples and were considered the dominant phyla. These included Firmicutes (med = 48.3%, range = [5.5, 97.4]), Bacteroidetes (med = 17.8%, range = [0, 63.0]), Actinobacteria (med = 14.6%, range = [0, 89.3]), and Proteobacteria (med = 0.94%, range = [0, 44.0]). Of the 586 species, 29 were present in at least 60% of individuals and at a relative abundance of at least 0.1% and were considered core (Fig. 13A, Appendix F). Species that were the most abundant included *Bacteroides uniformis* (med = 2.1%), *Collinsella aerofaciens* (med = 1.3%), and *Anaerostipes hadrus* (med = 1.2%). Species that were detected the most frequently among participants included *Ruthenibacterium lactatiformans* (prevalence = 99.5%), *Gordonibacter pamelaeae* (prevalence = 98.5%) and *Eggerthella lenta* (prevalence = 97.1%). Despite a core microbiome among participants, overall, microbiome compositions were highly dispersed (Fig. 13B), with a median distance to centroid of 0.13 (range = [0.002, 0.58]). Both core and non-core species contributed to this, with the relative abundance of core species ranging from 7.7 to 87.9%, (Fig. 13C).

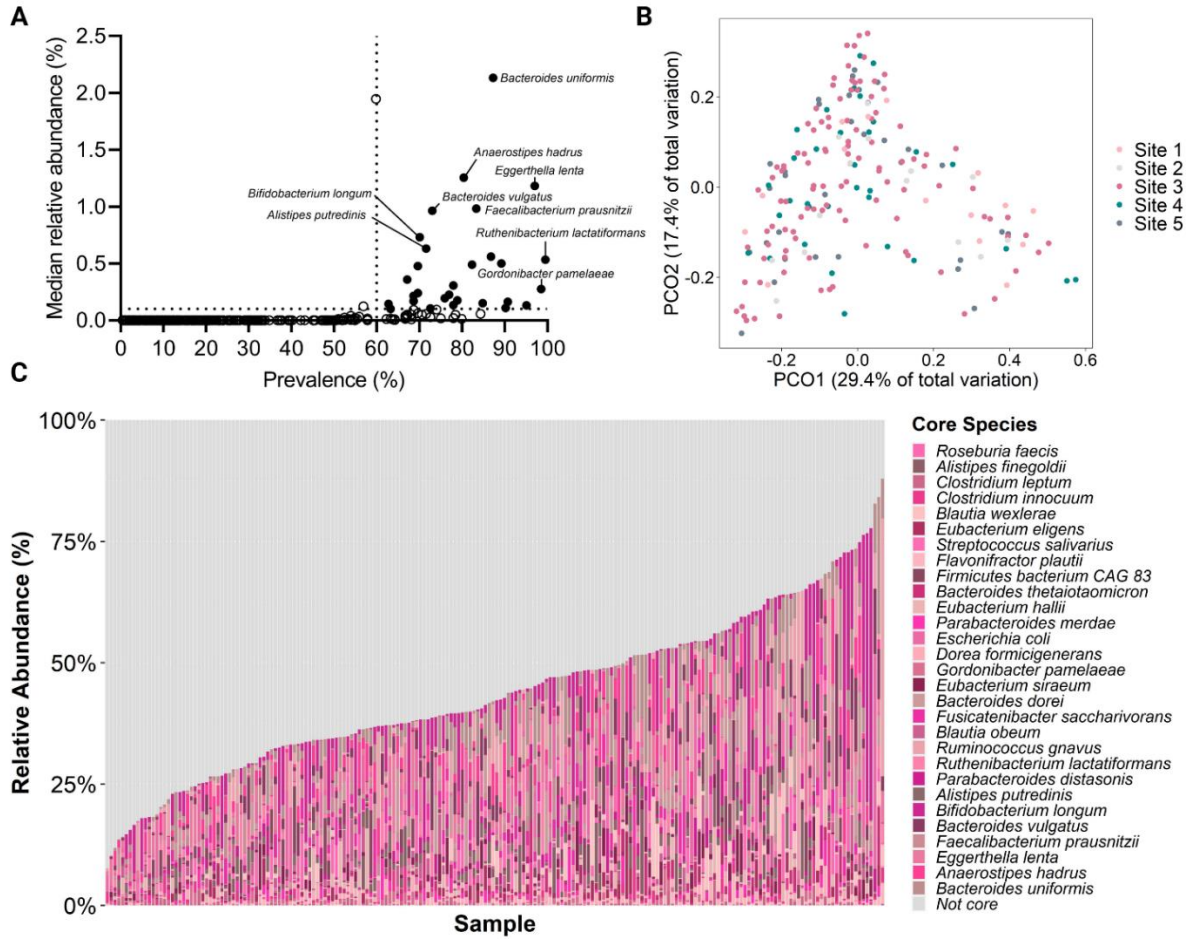


Figure 13. *Frequency of species detected in the stool microbiome compared to their relative abundances (A). Principle Coordinates Analysis (PCoA) plot showing dispersion of the stool microbiome among participants, where each dot represents an individual's microbiome relative to others (B). Taxa bar plot showing the distribution of 29 core species compared to non-core species in the stool microbiome of participants (C).*

3.2 Microbiome composition of the oropharynx

Metagenomic assessment of the oropharyngeal (OP) microbiome was available for 237 (94.0%) samples collected from the GRACE study. Across the OP sample, 10 unique phyla were detected, consisting of 90 genera or 333 different species. The median number of species detected per person was 49 (range = [3, 154]). Of the phyla detected in the OP microbiome for the cohort, 4 were present in 77.6% of samples and were considered dominant. Firmicutes were the most abundant (med = 61.7%, range = [2.6, 100]), followed by Actinobacteria (med = 18.4%, range = [0, 79.2]), Bacteroidetes (med = 7.5%, range = [0, 53.7]), and Proteobacteria (med = 0.6%, range = [0, 56.2]). Sixteen core genera, defined as those present in at least 60% of individuals and at a relative abundance of at least 0.1%, were identified in the OP microbiome of GRACE participants (Fig. 14A, Appendix F). Like the stool microbiome, overall, OP microbiome compositions were highly dispersed (Fig. 14B), with a median distance to centroid of 0.15 (range = [0.009, 0.45]). As per the stool samples, the relative abundance of all core species in the OP microbiome varied greatly and ranged from 0 to 99.5% (Fig. 14C). Species that were the most abundant in the OP microbiome included *Streptococcus salivarius* (med = 10.5%)*, Streptococcus parasanguinis* (med = 8.0%)*, and Veillonella atypica* (med= 2.3%). Species that were detected the most frequently among participants included *Streptococcus parasanguinis* (prevalence = 97.0%)*, Rothia mucilaginosa* (prevalence = 94.1%)*, and Streptococcus salivarius* (prevalence = 91.6%).

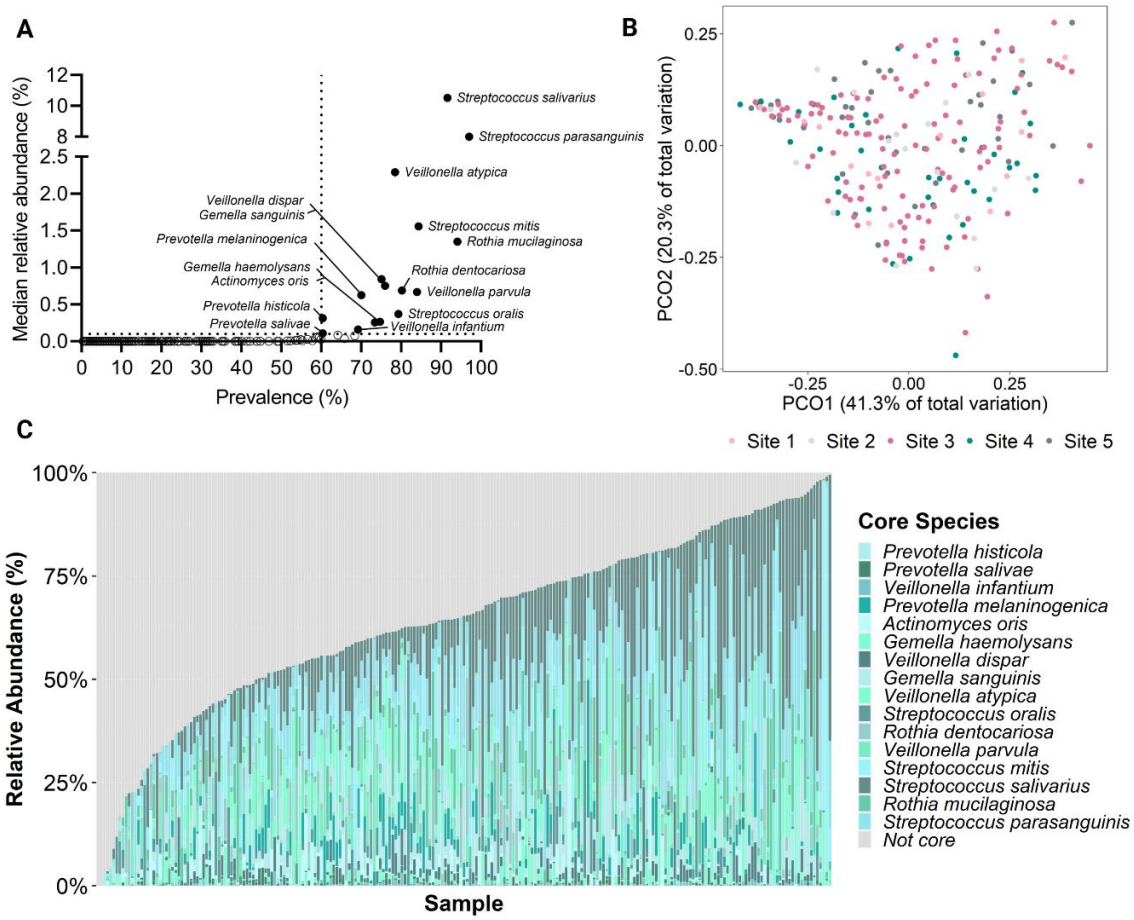


Figure 14. *Frequency of species detected in the OP microbiome compared to their relative abundances (A). Principle Coordinates Analysis (PCoA) plot showing dispersion of the OP microbiome among participants, where each dot represents an individual's microbiome relative to others (B). Taxa bar plot showing the distribution of 16 core species compared to non-core species in the OP microbiome of participants (C).*

3.3 Resistome composition of the stool

The resistome is the collection of antibiotic resistance genes (ARG) that are carried in our microbiome. ARG are complex, for example they can confer resistance via more than one mechanism and to more than one drug class. Presence of ARG does not necessarily indicate that the bacteria carrying it will be an MDRO. This report describes the resistome as a whole; clinical assessment of resistance will be addressed in later analysis.

A normalised count (rpkm) of stool metagenomic reads that aligned to the Comprehensive Antibiotic Resistance Database (CARD) was used to characterise the GRACE stool resistome. In total, 690 ARG were detected across 204 participants, which conferred resistance to 37 different classes of antibiotics (Fig. 15A). Most frequently, ARGs conferring resistance to macrolides (n=137 genes), tetracyclines (n=134 genes), cephalosporins (n=125 genes) and penicillins (n=120 genes) were identified in the stool resistome of GRACE participants. ARGs conferring resistance to macrolides (med = 1659.5 rpkm) and peptides (med = 1530.4 rpkm), however, were the most abundant in the resistome (Fig. 15A). The median number of ARGs carried per person was 375 (range = [291, 437]) (Fig. 15B). Genes carried in the stool microbiome of the GRACE cohort conferred resistance to antibiotics via 6 different mechanisms, including antibiotic inactivation (n=235, 33.8%) and antibiotic efflux (n=219, 31.5%; Fig. 15C).

These genes could be classified into 108 ARG families, which describe the function of each ARG. Families that made up the largest proportion of all genes detected included resistance-nodulation-cell division (RND) antibiotic efflux pumps (n=105, 15.2%), major facilitator superfamily (MFS) antibiotic efflux pumps (n=83, 12.0%), chloramphenicol acetyltransferases (n=29, 4.2%) and OXA beta-lactamases (n=27, 3.9%). The most abundant and frequently detected gene families were ATP-binding cassette (ABC) antibiotic efflux pumps (med = 1611.0 rpkm), RND antibiotic efflux pumps (med = 946.2 rpkm), and *vanR* genes (med = 743.0 rpkm; Fig. 15D).

3.4 Resistome composition of the oropharynx

Across 237 viable OP samples, a total of 424 ARGs were detected that conferred resistance to 38 different antibiotic drug classes. Genes conferring resistance to macrolides (n=118 genes), tetracyclines (n=107 genes), peptide antibiotics (n=97 genes), and phenicol antibiotics (n=85 genes) were the most common. Genes conferring resistance to macrolides (med = 1290.5 rpkm) and tetracyclines (med = 1033.4 rpkm) were the most abundant in the OP resistome (Fig. 16A). ARG richness per person was a median of 212 genes (range = [69, 315]) (Fig. 16B). Antibiotic efflux (n=189 genes) and target alteration (n=121 genes) were the most frequent of the 6 resistance mechanisms detected (Fig. 16C).

ARGs in the OP resistome could be classified into 68 different ARG families (Fig. 16D). RND antibiotic efflux pumps (n=84, 19.8%) and major facilitator superfamily (MFS) antibiotic efflux pumps (n=74, 17.5%) made up the greatest proportion of all genes detected in the OP resistome. ABC antibiotic efflux pump (median rpkm = 1647.6) and ABC ribosomal protection protein (median rpkm = 355.6) families were the most abundant.

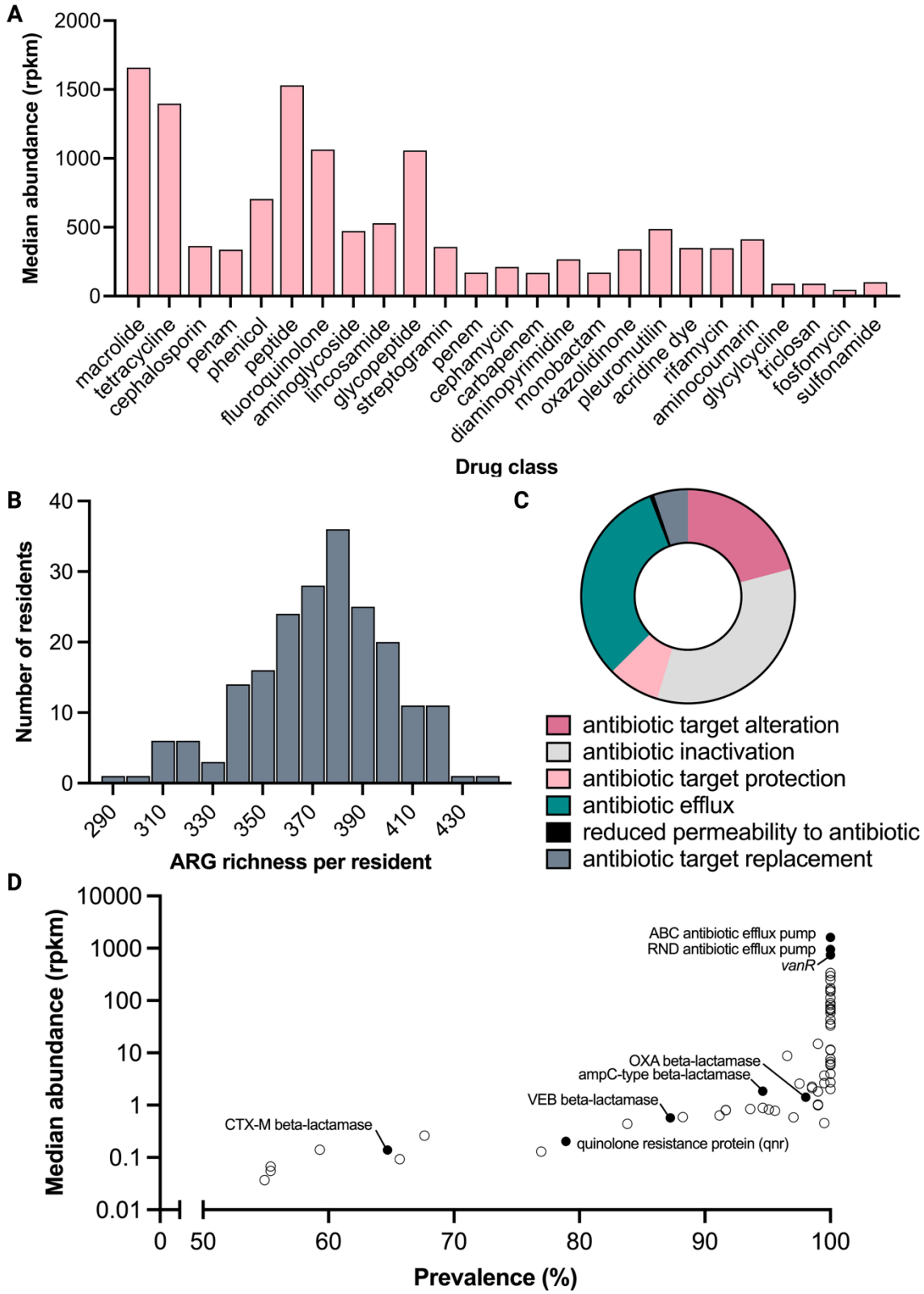


Figure 15. *Median abundance (rpkm) of ARG that confer resistance to antibiotic drug classes in the stool resistome (n=12 antibiotic classes not shown as they are extremely rare) (A). Distribution of stool ARG richness per person (B). Proportion of antibiotic resistance mechanisms observed in the stool resistome (C). Prevalence and abundance of ARG families in the stool resistome with most abundant and clinically important gene families labelled (n=47 families not shown as median abundance = 0 rpkm) (D). *ABC = ATP-binding cassette; RND= resistance-nodulation-cell division.*

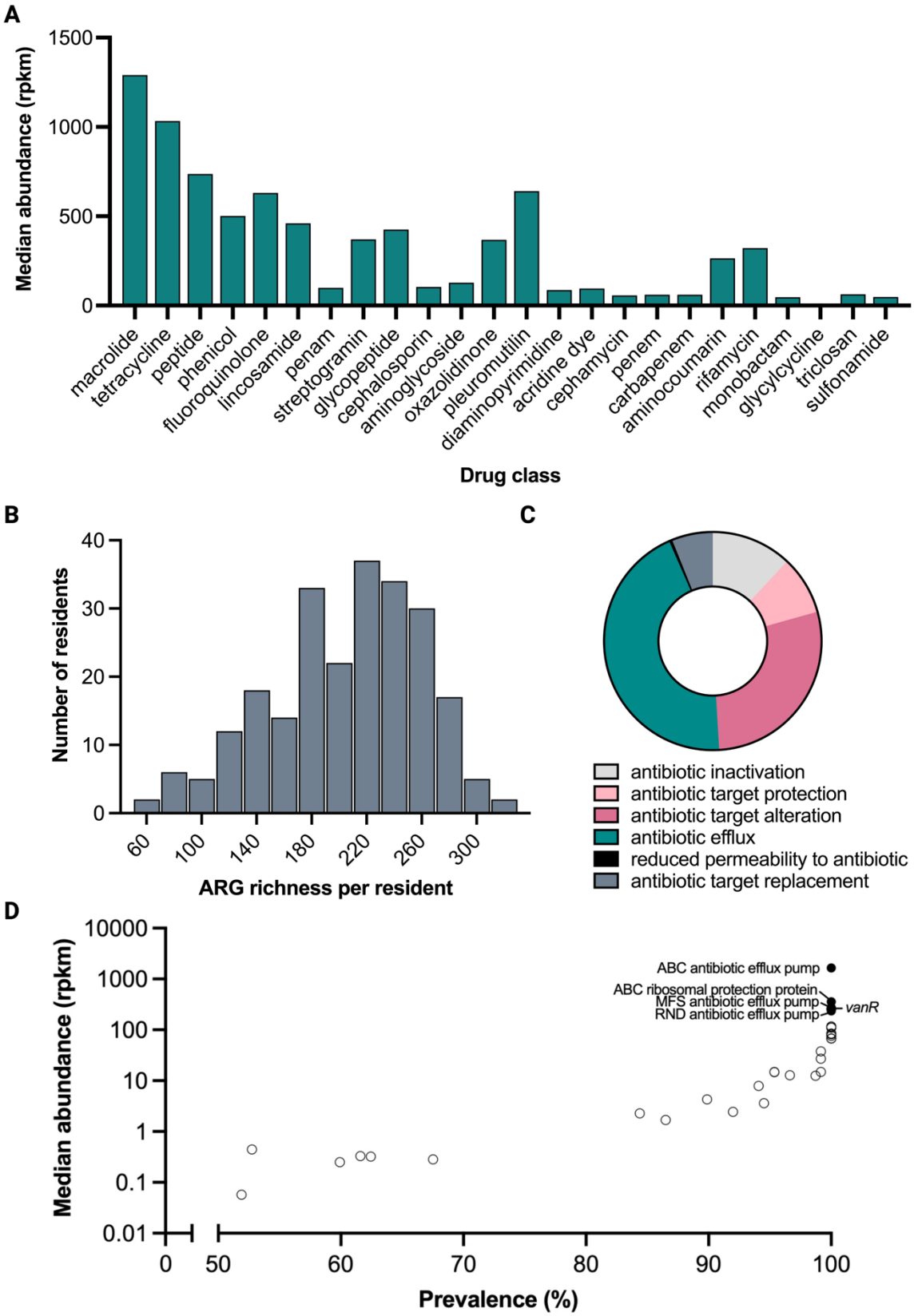


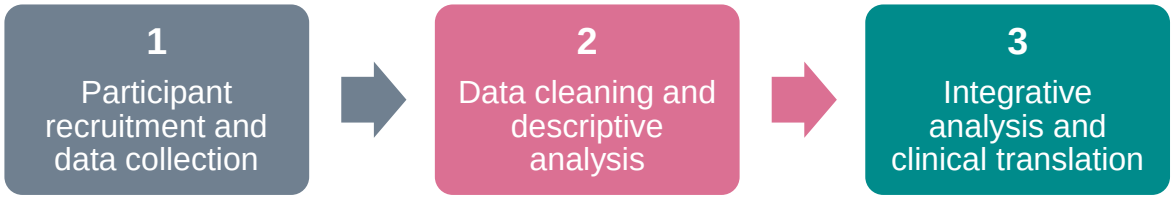
Figure 16. *Median abundance (rpkm) of ARG that confer resistance to antibiotic drug classes in the OP resistome (n=13 antibiotic classes not shown as they are extremely rare) (A). Distribution of OP ARG richness per person (B). Proportion of antibiotic resistance mechanisms observed in the OP resistome (C). Prevalence and abundance of ARG families in the OP resistome with most abundant gene families labelled (n=36 families not shown as median abundance = 0 rpkm) (D). *ABC= ATP-binding cassette; RND= resistance-nodulation-cell division; MFS= major facilitator superfamily.*

Conclusions

Described in this report are the clinical, environmental, and microbiological characteristics of participants of the GRACE study. Our cohort consists of permanent residents of south Australian, not-for-profit, metropolitan aged-care facilities. Participants were aged between 58 and 104 years, were mostly female and had been living in their current facility for between 3 and 5399 days. Most participants had a normal diet with standard supplementation. Over half of our cohort had a diagnosis of dementia and this was the same for a diagnosis of depression. Antibiotics were frequently used in this cohort, making up 3 of the 10 most commonly used medications. Around a third of all participants had a known hospital visit during the captured period. Large inter-individual variation in the microbiome and resistome compositions were observed and genes conferring resistance to macrolides and tetracyclines were common. As shown in this report, participants of the GRACE study were subjected to a number of exposures which we propose has a significant impact on the microbiome and resistome, and therefore risk of AMR carriage, transmission, and poor health outcomes.

Future Developments

In this report, we have presented the results from the first 2 stages of the GRACE study: 1) participant recruitment and data collection, and 2) data cleaning and descriptive analysis.



Going forward we will begin the third and final stage: 3) integrative analysis and clinical translation. We aim to use the data presented to here to determine how various exposures in the residential aged-care environment contribute to the acquisition and dispersal of AMR and answer the 5 research questions listed at the beginning of this document. We also aim to demonstrate that our study cohort is representative of those living in Australian aged-care facilities by publishing a cohort profile comparing our data to national averages. As a multidisciplinary team, we have the capacity to ensure that our findings are accessible and informative to other researchers, clinicians, as well as aged-care providers and policy makers.

List of Figures

List of Tables

Appendices

Appendix A: Methods

Ethics

Ethical approval for the study was obtained from the Southern Adelaide Clinical Human Research Ethics Committee (HREC/18/SAC/244). Participants provided written informed consent themselves or where third-party consent was required, a legal guardian or family member with power of attorney provided consent on their behalf.

Setting

Three aged-care providers agreed to participate for a total of 5 sites included in the study. Recruitment started at site 1 in March 2019 and was ceased during recruitment at site 5 due to the COVID-19 pandemic in March 2020. Data was collected at the end of recruitment for each site by the study team.

Recruitment of participants

All residents living in participating aged-care facilities at the time of recruitment were invited to join in the study. Participants were not eligible to consent if: 1) they were in respite care at the time of recruitment, 2) they were receiving palliative/end-of-life care, 3) it was recommended by management that they not be approached, and 4) we were unable to contact next of kin where third-party consent was required. In addition, some participants were unable to be approached due to the COVID-19 pandemic, which caused the study recruitment at the last site to cease early. Participants who required third-party consent were identified by the participating facility and communicated to the study team.

Data sources/measurements

Facility data and participant demographical data were obtained from the facility records. Details of other data collected is explained below.

<u>Aged Care Funding Instrument (ACFI) assessment.</u>

The ACFI constitutes a series of questions and data collection instruments which determine the level of care a person requires upon admission to a residential aged care facility, and therefore how much funding that facility requires to facilitate the required level of care. The ACFI focuses on care needs related to day-to-day and high frequency needs for care. Three domains of residential care are subsidised by the ACFI: activities of daily living, behaviour, and complex health care. The metrics of assessment range from A to D, with A requiring the lowest level of care, to D requiring the most. The individual assessments within these domains are assigned specific ratings and are detailed below.

Nutrition score: The level of assistance (independent OR supervision OR physical assistance) for tasks concerning readiness to eat (using utensils and cutting up/mixing food) and eating (putting food in mouth). A = No assistance, B = Supervision in one/both tasks or physical assistance in readiness to eat task, C = Supervision in one task and physical assistance in one task, D = Physical assistance with both tasks.
Mobility score: The level of assistance (independent OR supervision OR physical assistance) for tasks concerning transfer of position (wheelchair usage, moving from chairs to wheelchairs to beds, etc.), or locomotion (walking, pushing a wheelchair, attachment or passing of mobility aids such as prosthetic limbs or braces). A = No assistance, B = Supervision or physical assistance in one task, C = Supervision in one task and physical assistance in one task, D = Physical assistance with both tasks.

Personal hygiene score: The level of assistance (independent OR supervision OR physical assistance) for tasks concerning dressing and undressing, washing and drying, and grooming. A = No assistance, B = Supervision in one task, C = Physical assistance in one task, D = Physical assistance in all tasks.
Toileting score: The level of assistance (independent OR supervision OR physical assistance) for tasks concerning the use of a toilet (setting up to use the toilet), and toilet completion (the ability to appropriately manage the toileting activity). A = No assistance, B = Supervision in one task, C = Physical assistance in one task, D = Physical assistance in all tasks.
Continence score: The presence and/or frequency of urinary and faecal incontinence. A = No episodes of incontinence or self-manages continence devices, B = Incontinent of urine less than or equal to once per day, or faeces once or twice per week, C = 2-3 daily episodes of urinary incontinence/passing of urine during scheduled toileting, or 3-4 weekly episodes of faecal incontinence/passing faeces during scheduled toileting, D = More than 3 daily episodes of urinary incontinence/passing of urine during scheduled toileting, or more than 4 weekly episodes of faecal incontinence/passing of faeces during scheduled toileting.
Total domain activities of daily living score: The summarised assistance level required across all activities of daily living. A = Low, B = Medium, C = High.

Cognitive skills score: The level of impairment determined from the Psychogeriatric Assessment Scale – Cognitive Impairment Scale (PAS-CIS). A = No or minimal impairment, B = Mild impairment, C = Moderate impairment, D = Severe impairment.
Wandering score: Assessment of occurrence/frequency of problem wandering (repeated attempts to leave the service, or where presence is unwelcome or inappropriate). A = Problem wandering occurs less than 2 days per week, B = Problem wandering occurs at least 2 days per week, C = Problem wandering occurs at least 6 days in a week, D = Problem wandering occurs twice a day or more, at least 6 days in a week.
Verbal behaviour score: Assessment of verbal refusal of care, verbal disruption (not related to an unmet need), paranoid ideation that disturbs others, or verbal sexually inappropriate advances directed at another person. A = Verbal behaviour occurs less than 2 days per week, B = Verbal behaviour occurs at least 2 days per week, C = Verbal behaviour occurs at least 6 days in a week, D = Verbal behaviour occurs twice a day or more, at least 6 days in a week.
Physical behaviour score: Assessment of physical conduct by a resident that is threatening and has the potential to physically harm another person, socially inappropriate behaviour that impacts on other residents, and being constantly physically agitated. A = Physical behaviour occurs less than 2 days per week, B = Physical behaviour occurs at least 2 days per week, C = Physical behaviour occurs at least 6 days in a week, D = Physical behaviour occurs twice a day or more, at least 6 days in a week.
Depression score: Utilises the Cornell Scale for Depression (CSD) to evaluate symptoms associated with depression and dysthymia (chronic mood disturbance), and how these symptoms interfere with daily life. A = CSD score 0-8 and minimal or no symptoms, B = CSD score 9-13 and symptoms cause mild interference with daily function, C = CSD score = 14-18 and symptoms cause moderate interference with daily function, D = Diagnosed depression, CSD score 19-38, and symptoms majorly impact daily function.
Domain behavioural PAS CIS score: The level of impairment determined from the Psychogeriatric Assessment Scale – Cognitive Impairment Scale (PAS-CIS). Scale ranges from 0-21 (nil to severe impairment).
Total domain behavioural score: The summarised impact level of resident behaviour across all forms of physical and non-physical behaviour to determine total dependency on care. A = Low, B = Medium, C = High.

Medication score: The level of assistance required to take medication administered on a regular basis (including patches, oral administration, subcutaneous, intramuscular, and intravenous). A = No medication or self-manages medication, B = Application of patches at least weekly, or needs assistance with daily medication, C = Needs daily administration of a subcutaneous, intramuscular, or intravenous drug. Assessment prior to 2017 included an

option D for medication score. For these residents, scores A and B were the same, however there are some differences to the assignment of scores C and D. Specifically, C = Needs daily assistance with medications for between 6 and 11 minutes, and D = Needs greater than 11 minutes of daily assistance and/or administration of a subcutaneous, intramuscular, or intravenous drug.
Complex healthcare score: The assessed need for ongoing complex health care procedures and activities, with ratings relating to the technical complexity and frequency of the procedures. A = Score of 0 (no procedures), B = Score of 1-4 (assistance required with a low number of complex procedures), C = Score of 5-9 (assistance required with a moderate number of complex procedures), D = Score of 10+ (assistance required with a high number of complex procedures).
Total domain complex health care score: The summarised assistance level required across all forms of complex health care to determine total dependency on care. A = Low, B = Medium, C = High.

Mental and Behavioural Diagnoses
To support the ACFI assessment, residents are also evaluated for diagnosis of a neurological impairment which may influence their care requirements, as determined by the ACFI. These diagnoses include dementia, mood disorders, psychiatric and neurotic disorders, and evaluations of cognitive impairment. Counts and proportions of residents were calculated from ACFI data.

Comorbidities
Physical comorbidities and signs and symptoms were determined using the ACAP code system which is part of the ACFI assessment as described above. Any medical conditions listed in the ACFI are required to be supported by evidence from a medical professional.

PBS/MBS
De-identified DHS-linked data was received in May 2021. PBS data was checked against the information available in resident medication charts for 41 participants. Of 134 medication entries for the available period, expectations were met in 81.3% of instances. PBS and MBS data were cleaned to ensure 12 months of pre-enrolment data was available for each participant. Drug classes were grouped and classified according to their ATC code and health services were classified based on their assigned category.

Sample collection
Stool samples and oropharyngeal (OP) swabs were collected from consenting residents, as well as environmental swabs of participant rooms and communal areas. Swabs taken in participant rooms included bed remotes, overways, door handles and toilet flushes. Swabs taken in communal areas include staff room door handles, staff phones, staff computer keyboards, medication trolleys, dining tables, servery benches, public toilet seats, public toilet flushes, public toilet door handles, wheelchairs and mechanical lifters. Stool samples were collected in 20ml tubes with DNA stabilisation buffer (Norgen, ON, Canada) and swabs were collected and placed in a 2ml screw-cap tube contained 400µl of Tris-EDTA (TE) buffer (Invitrogen, CA, USA). All samples were stored at -80°C until processing.

Stool DNA extraction
DNA was extracted from stool samples using the Qiagen PowerLyzer PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Stool samples containing buffer were vortexed vigorously and 1 ml was transferred to a clean 2 ml tube. Samples were centrifuged for 20 min at 13,000 xg at 4°C and the supernatant was transferred

to a clean 2 ml screw-cap tube for storage. Faecal pellet was combined with 750 µl of bead solution and transferred to a glass bead tube. After adding 60 µl of cell lysis buffer, samples were incubated at 65°C for 10 min. Samples underwent bead-beating in a FastPrep®-24 Homogenizer (MP Biomedicals, CA, USA) for 2 pulses of 1 min at 6.5m/s and were centrifuged at 10,000 xg for 3 min at room temperature. Supernatant was discarded and 250 µl of Inhibitor Removal Technology® (IRT) was added and vortexed for 5 s. Samples were incubated at 4°C for 10 min, centrifuged for 3 min at 10,000 xg at room temperature, and 600 µl of supernatant was transferred to a clean 2 ml tube. Precipitation reagent was combined with the supernatant and the sample was centrifuged for 3 min at 10,000 xg at room temperature again before transfer of 750 µl supernatant to another clean 2 ml tube. High concentration salt solution was added to the supernatant and vortexed for 5 s. Then 675 µl of supernatant was added to a Spin Filter and centrifuged for 10,000 xg for 1 min at room temperature. Flow through was discarded and this step was repeated 2 more times. 500 µl of ethanol-based wash solution was added to the spin column and centrifuged at 10,000 xg for 1 min at room temperature. Flow through was discarded and residual ethanol wash solution was removed from the spin column by a second centrifuge at 10,000 xg for 1 min. Spin columns were transferred to a clean 2 ml tube, 50 µl of UltraPure RNAse DNAse-free water was added and centrifuged for 1 min 10,000 xg at room temperature and repeated 2 more times to collect all DNA. Eluted DNA was stored at -80°C until further processing.

Swab DNA extraction

DNA from swabs was extracted using the ZymoBIOMICS miniprep kit (Zymo Research, CA, USA). Swabs were spun down at 3374 x g for 5 min to collect all biological material and the resultant solution was added to a bead-beating tube containing 750 µl of lysis buffer. Samples underwent bead-beating for 1 min 5 times at a speed of 6.5m/s in a FastPrep®-24 Homogenizer (MP Biomedicals, CA, USA) for a total of 5 min with 5 min rest in between each run. Samples were centrifuged at 10,000 x g for 2 min then 700 µl was added to the III-F filter in a clean tube and centrifuged at 8,000 x g for 1 min. Filtered solution was transferred to a clean tube and 2100 µl of DNA binding buffer was added. Samples were vortexed vigorously then 800 µl of solution was added to a IICR filter and centrifuged at 10,000 x g for 1 min. Flow through was discarded and this process was repeated until all solution had been passed through the filter. After transferring the filter to a new tube, 400 µl of the first wash buffer was added to the filter and centrifuged at 10,000 x g for 1 min. Flow through was discarded then 700 µl of a second wash buffer was added to the filter, centrifuged at 10,000 x g for 1 min and a final 200 µl of wash buffer was added to ensure all wash buffer had passed through. Filters were transferred to a new clean tube and 100 µl of dH_2O at 60°C was added and incubated for 5 min. After centrifuging at 10,000 x g for 1 min, samples were added to a final spin column for purification and centrifuged at 16,000 x g for 3 min. For environmental swabs, the elution process was repeated 2 more times, then samples were concentrated and re-eluted in 75 µl of dH_2O. DNA was stored at -80°C until further processing.

Metagenomic sequencing

Stool samples and OP swabs of sufficient DNA quality underwent metagenomic sequencing. DNA fragmentation of samples was performed with Nextera XT DNA Library Prep Kit (Illumina, CA, USA). Samples were sequenced at a depth of 5Gb on an Illumina Novaseq platform with 150bp paired-end reads.

Bioinformatic processing

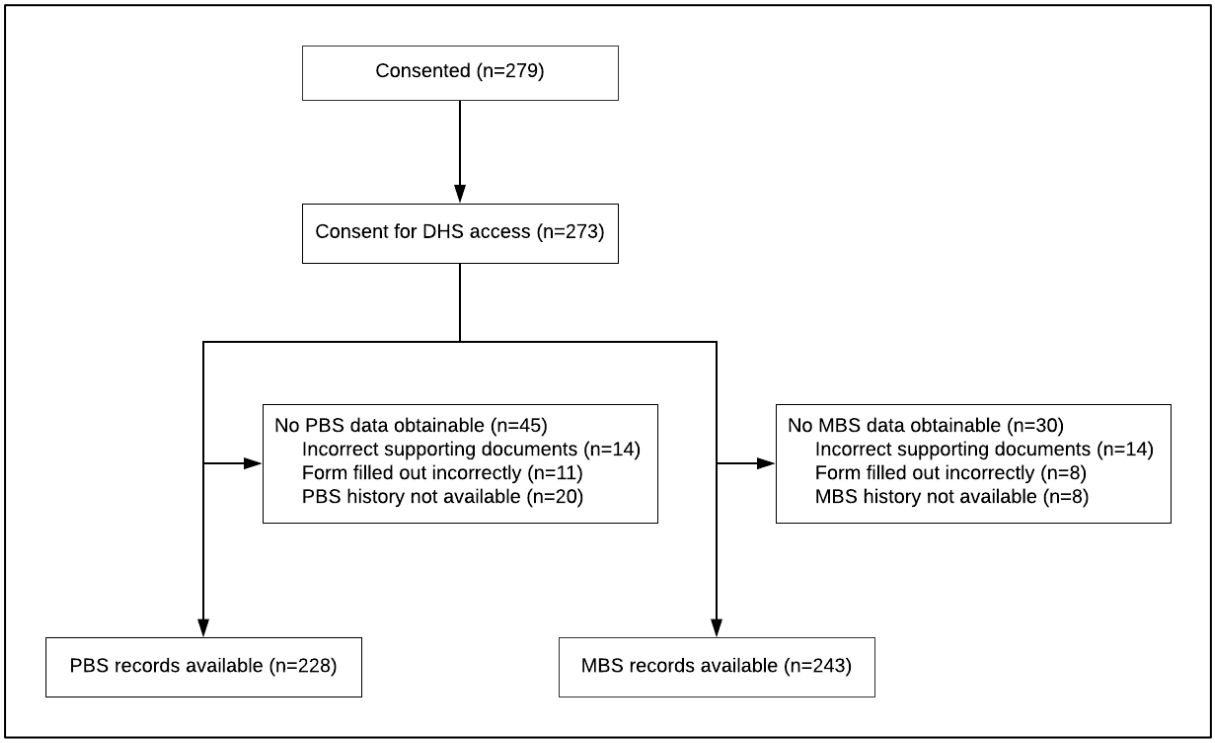
Paired-end sequences were quality filtered using Trimmomatic (version 0.39) and human reads were removed using Bowtie (version 2.3.5.1) using the NCBI human reference genome release GRCh38.[8, 9] Contigs were assembled de novo using IDBA-ud (version 1.1.3) and open reading frames were identified with MetaGeneMark (version 1.0).[10, 11] Non-redundant genes were extracted using CD-HIT (version 4.8.1) with parameters '-c 0.95 -aS 0.9' (genes with >95% identity and aligned length covering >90% of shorter gene) and genes less than 100 bp in length were removed.[12] A catalogue of 12,209,321 faecal genes and 2,334,932 OP genes were transcribed to amino acids using the European Molecular Biology Open Software Suite (EMBOSS v 6.6.0).[13] Transcribed genes were mapped to antimicrobial resistance genes in the Comprehensive Antibiotic Resistance Database (CARD) using BLASTP (version 2.9.0) with the following parameters: '-evalue 1e-10 -qcov_hsp_perc 99 -max_hsps 1 -max_target_seqs 1'.[14] Alignment of non-redundant gene catalogue with human-cleaned reads was performed with Bowtie (version 2.3.5.1).[9] Gene-length normalised read count calculation was performed and antimicrobial resistance gene quantification per sample was calculated in R (v4.0.2). Gene counts are reported as reads per kilobase of transcript, per million mapped reads (rpkm). Microbiome composition data was extracted from human-cleaned reads using MetaPhlAn (v3.0).[15]

Statistical methods

Raw data was cleaned and merged in Statistical Analysis Software (SAS) University Edition (v9.4) and exported for further processing. R (v4.0.2) and Prism (v9) was used for descriptive statistics and visualisation of data. Data was checked for normality and the appropriate metrics were reported depending on the outcome.

Appendix B: DHS data access

Availability of PBS and MBS data for GRACE participants and reasons where access was not possible.



Appendix C: Comorbidities

Full list of physical medical conditions and signs and symptoms with prevalence (number of residents) that affected the GRACE study cohort (classified by their ACAP categories).

Condition	ACAP code	N	%
Certain infectious and parasitic diseases			
Tuberculosis	0101	2	0.8
Poliomyelitis	0102	1	0.4
Diarrhoea and gastroenteritis of presumed infectious origin	0104	1	0.4
Unspecified/Unclassified infectious or parasitic disease	0199	5	1.9
Neoplasms (tumours/ cancers)			
Colorectal (bowel) cancer	0203	18	7.0
Lung cancer	0204	6	2.3
Skin cancer	0205	21	8.1
Breast cancer	0206	14	5.4
Prostate cancer	0207	17	6.6
Brain cancer	0208	2	0.8
Non-Hodgkin's lymphoma	0209	2	0.8
Leukaemia	0210	2	0.8
Other malignant tumours	0211	15	5.8
Other neoplasms	0299	12	4.7
Diseases of the blood and blood forming organs and immune mechanism			
Anaemia	0301	34	13.2
Immunodeficiency disorder (excluding AIDS)	0303	1	0.4
Other diseases of blood and blood forming organs and immune mechanism	0399	15	5.8
Endocrine, nutritional and metabolic disorders			
Disorders of the thyroid gland	0401	43	16.7
Diabetes mellitus–type 1 (IDDM)	0402	8	3.1
Diabetes mellitus–type 2 (NIDDM)	0403	52	20.2
Diabetes mellitus–other specified/unspecified/unable to be specified	0404	4	1.6
Malnutrition	0405	7	2.7
Nutritional deficiencies	0406	44	17.1
Obesity	0407	9	3.5
High cholesterol	0408	86	33.3
Other endocrine, nutritional and metabolic disorders	0499	22	8.5
Diseases of the nervous system			
Meningitis and encephalitis (excluding 'viral')	0601	1	0.4
Motor neurone disease	0603	2	0.8
Parkinson's disease	0604	24	9.3
Transient cerebral ischaemic attacks	0605	27	10.5
Brain disease/ disorders	0606	3	1.2
Multiple sclerosis	0607	3	1.2
Epilepsy	0608	7	2.7
Cerebral palsy	0610	2	0.8
Paralysis-non-traumatic	0611	8	3.1
Other diseases of the nervous system	0699	67	26.0
Diseases of the eye and adnexa			
Cataracts	0701	32	12.4
Glaucoma	0702	38	14.7
Blindness	0703	27	10.5
Poor vision	0704	41	15.9
Other diseases of the eye and adnexa	0799	31	12.0
Disease of the ear and mastoid process			
Ménière's disease	0801	11	4.3

Condition	ACAP code	N	%
Deafness/hearing loss	0802	69	26.7
Other diseases of the ear and mastoid process	0899	11	4.3
Diseases of the circulatory system			
Heart disease	0900	60	23.3
Angina	0903	6	2.3
Myocardial infarction	0904	18	7.0
Acute and chronic ischaemic heart disease	0905	41	15.9
Congestive heart failure	0906	45	17.4
Other heart diseases	0907	56	21.7
Cerebrovascular disease	0910	6	2.3
Subarachnoid haemorrhage	0911	2	0.8
Intracerebral haemorrhage	0912	2	0.8
Other intracranial haemorrhage	0913	5	1.9
Cerebral infarction	0914	5	1.9
Stroke (CVA)	0915	45	17.4
Other cerebrovascular diseases	0916	11	4.3
Other diseases of the circulatory system	0920	9	3.5
Hypertension	0921	174	67.4
Hypotension	0922	18	7.0
Abdominal aortic aneurysm	0923	5	1.9
Other arterial or aortic aneurysms	0924	5	1.9
Atherosclerosis	0925	3	1.2
Other diseases of the circulatory system n.e.s	0999	35	13.6
Diseases of the respiratory system			
Influenza and pneumonia	1002	15	5.8
Acute lower respiratory infections	1003	8	3.1
Other diseases of the respiratory system	1004	14	5.4
Chronic lower respiratory diseases	1005	56	21.7
Other diseases of upper respiratory tract	1099	7	2.7
Diseases of the digestive system			
Diseases of the intestine	1101	103	39.9
Diseases of the peritoneum	1102	2	0.8
Diseases of the liver	1103	7	2.7
Other diseases of the digestive system	1199	101	39.1
Diseases of the skin and subcutaneous tissue			
Skin and subcutaneous tissue infections	1201	18	7.0
Skin allergies	1202	31	12.0
Other diseases of the skin and subcutaneous tissue	1299	34	13.2
Diseases of the musculoskeletal system and connective tissue			
Rheumatoid arthritis	1301	8	3.1
Other arthritis and related disorders	1302	213	82.6
Deformities of joints/ limbs–acquired	1303	9	3.5
Back problems–dorsopathies	1304	32	12.4
Other soft tissue/ muscle disorders	1305	17	6.6
Osteoporosis	1306	88	34.1
Other disorders of the musculoskeletal system and connective tissue	1399	39	15.1
Diseases of the genitourinary system			
Kidney and urinary system (bladder) disorders	1401	59	22.9
Urinary tract infection	1402	46	17.8
Stress/urinary incontinence	1403	180	69.8
Other diseases of the genitourinary system	1499	34	13.2
Congenital malformations, deformations and chromosomal abnormalities			
Down's syndrome	1503	1	0.4
Other chromosomal abnormalities	1504	1	0.4
Other congenital malformations and deformations	1599	1	0.4
Injury, poisoning and certain other consequences of external causes			

Condition	ACAP code	N	%
Injuries to the head	1601	5	1.9
Injuries to arm/hand/shoulder	1602	20	7.8
Injuries to leg/knee/foot/ankle/ hip	1603	23	8.9
Amputation of the finger/thumb/hand/arm/shoulder–traumatic	1604	2	0.8
Amputation of toe/ankle/foot/leg–traumatic	1605	9	3.5
Fracture of neck	1606	6	2.3
Fracture of rib(s), sternum and thoracic spine	1607	18	7.0
Fracture of lumbar spine and pelvis	1608	26	10.1
Fracture of shoulder, upper arm and forearm	1609	15	5.8
Fracture at wrist and hand level	1610	7	2.7
Fracture of femur	1611	33	12.8
Fracture of lower leg and foot	1612	5	1.9
Other injury, poisoning and consequences of external causes	1699	8	3.1
Symptoms and signs n.o.s or n.e.s			
Breathing difficulties/ shortness of breath	1703	17	6.6
Pain	1704	118	45.7
Nausea and vomiting	1705	5	1.9
Dysphagia	1706	29	11.2
Bowel/faecal incontinence	1707	98	38.0
Unspecified urinary incontinence	1708	26	10.1
Retention of urine	1709	1	0.4
Jaundice (unspecified)	1710	1	0.4
Disturbances of skin sensation	1711	2	0.8
Rash and other nonspecific skin eruption	1712	3	1.2
Abnormal involuntary movements	1713	3	1.2
Abnormalities of gait and mobility	1714	53	20.5
Falls (frequent with unknown aetiology)	1715	119	46.1
Confusion	1716	31	12.0
Amnesia	1717	22	8.5
Dizziness and giddiness	1718	15	5.8
Restlessness and agitation	1719	4	1.6
Irritability and anger	1721	4	1.6
Speech and voice disturbances	1725	10	3.9
Headache	1726	4	1.6
Malaise and fatigue	1727	32	12.4
Blackouts, fainting, convulsions	1728	3	1.2
Oedema (not specified)	1729	107	41.5
Symptoms and signs concerning food and fluid intake	1730	29	11.2
Other symptoms and signs	1799	1	0.4
Other health condition not elsewhere specified	1899	82	31.8

n.e.s. = not elsewhere specified; n.o.c. = not otherwise classified

Appendix D: Most prevalent medications

Top 10 most prevalent medications used by the GRACE cohort in the 12 months prior to enrolment in the study. N refers to the number of residents who were supplied each medication at least once.

Medication	ATC Code	N (%)
Macrogol	A06AD15	82 (36.0)
Furosemide	C03CA01	76 (33.3)
Pantoprazole	A02BC02	69 (30.3)
Cefalexin	J01DB01	64 (28.1)
Hypromellose and carboxymethylcellulose (eye drops/gel)	S01XA20	57 (25.0)
Amoxicillin and clavulanic acid	J01CR02	50 (21.9)
Paracetamol	N02BE01	50 (21.9)
Denosumab	M05BX04	46 (20.2)
Trimethoprim	J01EA01	44 (19.3)
Oxycodone	N02AA05	42 (18.4)

Appendix E: MBS codes and usage

List of healthcare services accessed by the GRACE cohort in the 12 month prior to enrolment (classified MBS category descriptions). N refers to the number of residents who accessed the service at least once.

MBS category description	MBS category code	N (%)
Professional attendances		
General practitioner attendances to which no other item applies	A1	94 (38.7)
Specialist attendances to which no other item applies	A3	77 (31.7)
Consultant psychiatrist attendances to which no other item applies	A8	6 (2.5)
Urgent attendance after hours	A11	150 (61.7)
Health assessments by general practitioners	A14	157 (64.6)
General practitioner management plans, team care arrangements, multidisciplinary care plans	A15	197 (81.1)
Domiciliary and residential management reviews	A17	150 (61.7)
Attendances by medical practitioners who are emergency physicians (private only)	A21	9 (3.7)
General practitioner after-hours attendances to which no other item applies	A22	207 (85.2)
Attendance by specialist in geriatric medicine	A28	26 (10.7)
Diagnostic imaging services		
Ultrasound	I1	71 (29.2)
Computerised tomography	I2	59 (24.3)
Diagnostic radiology	I3	99 (40.7)
Nuclear medicine	I4	6 (2.5)
Magnetic resonance imaging	I5	13 (5.3)
Pathology services		
Haematology services	P1	167 (68.7)
Chemical services	P2	204 (84.0)
Microbiology services	P3	195 (80.3)
Immunology services	P4	27 (9.9)
Tissue pathology	P5	33 (13.6)
Cytology services	P6	6 (2.5)
Genetic tests	P7	3 (1.2)
Simple basic pathology tests	P9	2 (0.8)
Patient episode initiation	P10	226 (93.0)
Specimen referred testing	P11	3 (1.2)
Therapeutic services		
Surgical operative services	T8	61 (25.1)
Miscellaneous services		
Allied health services	M3	155 (63.8)

Appendix F: Core stool and oropharyngeal taxa

Prevalence and relative abundance of species identified as core in the stool and oropharyngeal microbiome of GRACE participants.

Species name	Prevalence %	Median abundance (range), %
Stool		
Roseburia faecis	63.2	0.10 (0, 30.9)
Alistipes finegoldii	72.5	0.10 (0, 17.5)
Clostridium leptum	90.2	0.12 (0, 4.7)
Clostridium innocuum	95.1	0.13 (0, 13.3)
Blautia wexlerae	77.9	0.13 (0, 16.6)
Eubacterium eligens	62.7	0.14 (0, 13.6)
Streptococcus salivarius	84.8	0.15 (0, 13.9)
Flavonifractor plautii	90.7	0.16 (0, 6.4)
Firmicutes bacterium CAG 83	68.6	0.17 (0, 13.5)
Bacteroides thetaiotaomicron	78.9	0.18 (0, 12.2)
Eubacterium hallii	76.0	0.20 (0, 12.5)
Parabacteroides merdae	68.6	0.22 (0, 10.5)
Escherichia coli	77.0	0.23 (0, 43.6)
Dorea formicigenerans	69.6	0.24 (0, 6.3)
Gordonibacter pamelaeae	98.5	0.28 (0, 5.4)
Eubacterium siraeum	77.9	0.31 (0, 10.3)
Bacteroides dorei	67.2	0.36 (0, 22.6)
Fusicatenibacter saccharivorans	69.6	0.48 (0, 12.3)
Blautia obeum	82.4	0.49 (0, 8.4)
Ruminococcus gnavus	89.2	0.50 (0, 57.4)
Ruthenibacterium lactatiformans	99.5	0.53 (0, 15.0)
Parabacteroides distasonis	86.8	0.56 (0, 13.4)
Alistipes putredinis	71.6	0.63 (0, 7.4)
Bifidobacterium longum	70.1	0.73 (0, 60.2)
Bacteroides vulgatus	73.0	0.96 (0, 31.2)
Faecalibacterium prausnitzii	83.3	0.98 (0, 19.0)
Eggerthella lenta	97.1	1.2 (0, 18.9)
Anaerostipes hadrus	80.4	1.3 (0, 34.4)
Bacteroides uniformis	87.3	2.1 (0, 28.2)
Oropharyngeal		
Prevotella salivae	60.3	0.11 (0, 4.1)
Veillonella infantium	69.2	0.16 (0, 3.4)
Actinomyces oris	73.4	0.26 (0, 29.9)
Gemella haemolysans	74.7	0.27 (0, 40.5)
Prevotella histicola	60.3	0.31 (0, 31.1)
Streptococcus oralis	79.3	0.37 (0, 54.7)
Prevotella melaninogenica	70.0	0.63 (0, 33.4)
Veillonella parvula	84.0	0.67 (0, 42.0)
Rothia dentocariosa	80.2	0.69 (0, 64.0)
Gemella sanguinis	75.9	0.75 (0, 17.2)
Veillonella dispar	75.1	0.84 (0, 21.9)
Rothia mucilaginosa	94.1	1.4 (0, 57.8)
Streptococcus mitis	84.4	1.6 (0, 93.4)
Veillonella atypica	78.5	2.3 (0, 32.0)
Streptococcus parasanguinis	97.0	8.0 (0, 45.9)
Streptococcus salivarius	91.6	10.5 (0, 70.0)

Appendix G: Data completeness

Data completeness in the GRACE dataset varies by data source and individual variables. We have presented the availability of data for variables reported throughout the text in the below table.

Data Item	Availability % (N)
Demographics	
Age	100 (279)
Sex	100 (279)
Memory support room	100 (279)
Single or shared room	100 (279)
Medical needs	
Urinary catheter *in situ*	100 (279)
Urostomy	100 (279)
Vascular catheter *in situ*	100 (279)
Tracheostomy	100 (279)
Colostomy/ileostomy	100 (279)
Wound care (type)	99.3 (277)
Carriage of MDRO	100 (279)
Diet	
Diet type	99.6 (278)
Meal texture	100 (279)
Liquid texture	100 (279)
Prescribed supplements	96.8 (270)
ACFI: ADL	
Nutrition	98.2 (274)
Mobility	98.2 (274)
Personal hygiene	98.2 (274)
Toileting	98.2 (274)
Continence	97.8 (273)
Total ADL score	98.9 (276)
ACFI: Behaviour	
Cognitive skills	98.2 (274)
Wandering	98.2 (274)
Verbal behaviour	98.2 (274)
Physical behaviour	98.2 (274)
Depression	98.2 (274)
PAS-CIS score	51.3 (143)
Total behaviour score	97.5 (272)
ACFI: CHC	
Medication	98.2 (274)
Complex healthcare	98.2 (274)
Total CHC score	98.9 (276)
ACFI: Mental and behavioural diagnoses	
Mental and behavioural diagnoses	100 (279)
Impairment level	98.2 (274)
ACFI: Comorbidities	
ACAP diagnosis codes	92.5 (258)
DHS-linked data	
PBS	81.7 (228)
MBS	87.1 (243)
Sample availability for microbiome and resistome composition	
Oropharyngeal swab	84.9 (237)
Stool sample	73.1 (204)
Both	69.5 (194)

References

1. Australian Bureau of Statistics. *Australian Demographic Statistics, Dec 2016* (accessed 15/08/17); Available from: http://www.abs.gov.au/.
2. Australian Bureau of Statistics. *Housing assistance: home care, hostels and nursing homes* (accessed 25/09/17); Available from: http://www.abs.gov.au.
3. The Royal Australian College of General Practitioners. *Medical care of older persons in residential aged care facilities 4th ed*. 2006 (accessed 25/09/17); Available from: http://www.racgp.org.au
4. Ricchizzi, E., et al., *Antimicrobial use in European long-term care facilities: results from the third point prevalence survey of healthcare-associated infections and antimicrobial use, 2016 to 2017.* Eurosurveillance, 2018. **23**(46): p. 1800394.
5. World Health Organisation, *The evolving threat of antimicrobial resistance - options for actions*. 2012, WHO: Geneva.
6. Pop-Vicas, A.E. and E.M. D'Agata, *The rising influx of multidrug-resistant gram-negative bacilli into a tertiary care hospital.* Clinical infectious diseases, 2005. **40**(12): p. 1792-1798.
7. Masnoon, N., et al., *What is polypharmacy? A systematic review of definitions.* BMC Geriatrics, 2017. **17**(1): p. 230.
8. Bolger, A.M., M. Lohse, and B. Usadel, *Trimmomatic: a flexible trimmer for Illumina sequence data.* Bioinformatics, 2014. **30**(15): p. 2114-2120.
9. Langmead, B. and S.L. Salzberg, *Fast gapped-read alignment with Bowtie 2.* Nature methods, 2012. **9**(4): p. 357-359.
10. Peng, Y., et al., *IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth.* Bioinformatics, 2012. **28**(11): p. 1420-1428.
11. Besemer, J. and M. Borodovsky, *GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses.* Nucleic acids research, 2005. **33**(suppl_2): p. W451-W454.
12. Fu, L., et al., *CD-HIT: accelerated for clustering the next-generation sequencing data.* Bioinformatics, 2012. **28**(23): p. 3150-3152.
13. Rice, P., I. Longden, and A. Bleasby, *EMBOSS: the European molecular biology open software suite.* 2000.
14. Alcock, B.P., et al., *CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database.* Nucleic acids research, 2020. **48**(D1): p. D517-D525.
15. Beghini, F., et al., *Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3.* eLife, 2021. **10**: p. e65088.