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Prevalence of extended-spectrum β-lactamase-producing Enterobacterales and carbapenemase-resistant Enterobacterales in British military cohorts

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ABSTRACT

Introduction Travel to resource-limited settings is a known risk for acquisition of extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE), which are both associated with increased morbidity and mortality. We investigated the ESBL-PE and CRE baseline prevalence in British service personnel (SP).

Methods SP provided faecal samples for research projects in several different settings, between September 2021 and April 2022. Bacterial colonies from faecal isolates were recovered from incubated ChromID ESBL plates (bioMérieux, Marcy-l'Étoile, France) and DNA extracted using Qiagen DNeasy extraction kits (Qiagen, UK). PCR to identify β-lactamase and CRE encoding genes was performed using the Rotor-Gene Q (RGQ) (Qiagen, UK), with positivity detected by RGQ software. Phenotypic assessment of antimicrobial susceptibility was not performed.

Results Out of 250 personnel approached, 239 (85.5% men, median (IQR) age 31 (26–37) years) provided faecal samples suitable for analysis. The ESBL prevalence was 40/239 (16.7%), with ESBL-producing *Escherichia coli* detected in 39 (16.3%) samples and ESBL-producing *Klebsiella pneumoniae* in 1 (0.4%) sample. Combinations including Temoniera, sulfhydryl reagent variable (SHV), cefotaxime hydrolysing β-lactamase (Munich) (CTX-M) 1 and CTX-M 9 genes were detected in 18 (7.5%), 33 (13.8%) 16 (6.7%) and 8 (3.3%) samples, respectively. *E. coli* samples had mixtures of all four genotypes with SHV predominating. One (0.4%) sample carried all four gene types and the only *K. pneumoniae* sample carried a single SHV gene. No CRE were detected.

Conclusions The prevalence of ESBL-PE in cohorts of SP closely matches that of civilian populations in England; however, we noted differences in ESBL genotype distribution. Potential exposure risks for SP from international travel and occupational trauma emphasise the need for repeated surveillance to characterise and detect changes in acquisition epidemiology and carriage of ESBL. Such prospective data have important antimicrobial stewardship implications in optimising clinical outcomes, controlling resistance and guiding empirical antibiotic formulary policy recommendations.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Multidrug-resistant Gram-negative bacteria (MDR-GNB) continue to compromise the efficacy of antibiotics due to their ability to evolve rapidly.
⇒ There is a paucity of data on gastrointestinal MDR-GNB carriage in British military populations.

WHAT THIS STUDY ADDS

⇒ Prevalence rates of extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) in British service personnel are similar to the general UK population, but the distribution of genotypes found in this study differs.
⇒ Characterisation of ESBL-PE and data on their geographical distribution is vital to inform recommendations for deployed empirical antimicrobial formulary, particularly for penetrating abdominal trauma.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Sustained surveillance of MDR-GNB in highly mobile military populations is important to identify associations between genotypic distribution and deployment destinations.

INTRODUCTION

The global spread of multidrug-resistant Gram-negative bacteria (MDR-GNB) is a significant public health threat.[1 2] MDR-GNB can be community acquired and/or healthcare associated and are linked to increased morbidity and mortality.[3] Enterobacterales are a large order of Gram-negative pathogens including *Escherichia coli* and *Klebsiella pneumoniae*.[1] The most recent surveillance of surgical site infections (SSIs) in English National Health Service hospitals in 2022 and 2023 found that Enterobacterales continued to account for the highest proportion of causative organisms across all surgical categories for both superficial (32.6%) and deep (26.8%) SSIs.[4] Changes in healthcare policies around MDR-GNB surveillance, as well as modern diagnostic techniques and antimicrobial stewardship, can improve detection and overall patient outcomes.[2 5]

The most frequently encountered resistance mechanism observed in MDR-GNB is the production of extended-spectrum β-lactamase (ESBL) enzymes that degrade β-lactam rings in penicillin, aminopenicillin, aztreonam and first-generation,

second-generation and third-generation cephalosporins,[2 6] although they are hindered by β-lactamase inhibitors such as clavulanic acid.[6] The three main ESBL classes are Temoniera (TEM), sulfhydryl reagent variable (SHV) and cefotaxime hydrolysing β-lactamase (Munich) (CTX-M), which is the most prevalent in the UK and worldwide.[5 7] ESBL-producing *E.coli* have been found in 11% of faecal samples across England, Scotland and Wales.[8] English surveillance programme for antimicrobial utilisation and resistance (ESPAUR) data consistently show *E. coli* (irrespective of antimicrobial sensitivity patterns) to be the most frequently reported cause of monomicrobial blood stream infection (20.9%).[9]

Acquisition of carbapenemase resistance mechanisms can further challenge treatment of clinically relevant Enterobacterales infections. Carbapenem-resistant Enterobacterales (CRE) are difficult to treat, as they have developed more widespread resistance to carbapenems[1] by production and release of carbapenem- hydrolysing β-lactamase enzymes or structural mutations.[10] Carbapenemases include the serine-based enzymes such as *K. pneumoniae* carbapenemase, oxacillinase-like carbapenemases and the metallo-β-lactamases, including Verona Integron-encoded metallo-β-lactamase, imipenemase and New Delhi Metallo β-lactamase.[11] CRE are a significant cause of healthcare-associated infections. Transfer of resistance genes readily occurs within the human gastrointestinal tract,[12] facilitating transmission from person to person.[13] Recent reports show that the rates of acquired carbapenemase-producing organisms in England varied according to region: London (2.4 episodes per 100 000 population), followed by the North West (2.2 episodes per 100 000 population), then the South West region (0.2 per 100 000 population).[14]

Antimicrobial resistance (AMR) is an increasing problem related to armed conflict and increases mortality and morbidity in the wounded.[15] Data on post-travel colonisation with extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) including assessment of associated predictors for sustained carriage and onward transmission are very limited.[16] Recent preliminary findings from a report on British service personnel (SP) deployed to Kenya showed a 26.5% ESBL-PE baseline prevalence.[17] Studies involving Polish and French personnel deployed to Afghanistan have shown even higher rates of acquisition of ESBL-PE of 70% and 88%, respectively.[18 19] Lower colonisation rates of 4.7% have been described in German soldiers when faecal samples were analysed 8–12 weeks after returning from predominantly subtropical or tropical deployment locations.[20] In contrast, the prevalence of CRE among wounded US SP has reportedly been low (0.4%), but emphasis has been placed on the difficulty of making any conclusions regarding association between carbapenem resistance, antibiotic exposure and clinical outcomes.[21]

We studied faecal samples collected from different cohorts of British military personnel with the primary aim of determining the baseline prevalence of ESBL-PE and CRE carriage rates. Our secondary aim was to identify AMR genes and characterise β-lactamase and carbapenem encoding genes from these faecal samples

METHODS

Timelines

Faecal samples were obtained from British military personnel separated into groups according to regimental system, then into cohorts according to deployment dynamics.

Samples were collected in the UK from groups 1 (battlegroup deploying to Kenya) and 4 (infantry battalion deploying to Mali) (n=34) between January and July 2022, and from groups 5 (infantry company in the UK), 6 (infantry company deploying to Belize), 7 (combat support unit in the UK) and 9 (infantry company post deployment to Belize and Brunei) (n=113) between September 2021 and February 2022. Samples from groups 2 (medical regiment squadron in Kenya) and 3 (infantry company in Kenya and deploying to various countries within Sub-Saharan Africa) (n=29) were collected in Nanyuki, Kenya, within 5 days of arrival between March and April 2022. These groups were all combined as the predeployment cohort (Table 1). Deployment samples were collected from group 8 (group 1 battlegroup SP who developed TD in Kenya) (n=63) in Nanyuki, Kenya, during a previously described diarrhoea outbreak.[22] The smaller postdeployment cohort (group 9) provided samples after travel to Belize or Brunei. There were insufficient sequential samples from individuals in any of the cohorts for comparisons to identify ESBL-PE acquisition time-points.

Table 1 Sample collection group profiles according to destination country and collection time-points

Cohort	Group	Group description	Destination	Sample collection	N (%)
1 (Pre deployment)	1	[1]BG	Kenya	UK	24 (10%)
	2	[2]Med Sqn	Kenya	Kenya	21 (8.8%)
	3	[3]Inf Coy STTT	Sub-Saharan Africa	Kenya	8 (3.3%)
	4	[4]Inf Bn	Mali	UK	10 (4.2%)
	5	[5]Inf Coy (A)	Not deployed	UK	56 (23.4%)
	6	[5]Inf Coy (B)	Belize	UK	19 (7.9%)
	7	[6]Combat Support	Not deployed	UK	19 (7.9%)
					157(65.6%)
2 (During deployment)	8	[7]BG	Kenya	Kenya	**63 (26.4%)**
3 (Post deployment)	9	[5]Inf Coy (C)	Belize, Brunei	UK	**19 (7.9%)**

Individual groups are arranged into deployment cohorts (n=sample size) with deployment location and sample collection location provided.
[1]BG = battlegroup; [2]Med Sqn = medical squadron; [3]Inf Coy STTT = infantry company short term training team; [4]Inf Bn = infantry battalion; [5]Inf Coy A–C = infantry companies A–C; [6]Combat Support = non infantry soldiers from various corps; [7]BG = group of soldiers who developed travellers' diarrhoea while in Kenya.

Sample collection and storage

Samples were self-collected in Hystool (Hystool, UK) faecal collection bags or other appropriate sterile repositories by participants, who then transferred approximately 10 mL of faeces into 30 mL sterile bottles marked with participant unique identifiers. These samples were picked up from prearranged points by investigators, aliquoted into 1 mL cryovials without preservative and stored at −80°C. Samples that could not be collected in person from participants based in the UK were sent by mail at ambient temperature and without preservatives, to arrive at the Liverpool School of Tropical Medicine within 48 hours of collection. These samples were frozen without preservative at −80°C in their collection bottles.

Faecal sample solution preparation

187.5 g maltodextrin and 62.5 g trehalose were added to 1 L phosphate-buffered saline and a stir bar added to the bottle. This was placed on a magnetic heat block, then heated to around 40°C until dissolved. 100 mL of solution were then removed and replaced with 100 mL glycerol to give a final concentration of 10% glycerol. This was placed on a magnetic stirrer to mix the solution, which was sterilised by vacuum filtration.

Bacterial recovery and plating

For samples in 1 mL collection tubes, stool solution was added to the sample up to a volume of 500 µL. Smaller measure sample extraction without thawing first was impracticable and would probably have impacted bacterial recovery. For samples in 30 mL tubes, 5 mL was transferred into 15 mL tubes using a cotton swab, and 1 mL of stool solution was added. All samples were placed in a water bath at 37°C, left for 5 min then vortex mixed before being returned to the water bath for a further minute. For each of the samples, 10 µL of solution was applied to ChromID ESBL plates (bioMérieux, Marcy-l'Étoile, France) and samples streaked on individual plate surfaces. Plates were incubated in aerobic conditions at 37°C for 48 hours, after which they were inspected for colony growth. Suitable colonies were then suspended in Microbank bead tubes and stored at −20°C.

DNA isolation and extraction

Genomic DNA was extracted from a single colony of each isolate, suspended in 1 mL of sterile water, using a Qiagen DNeasy extraction kit following the manufacturer's protocol for Gram-negative bacterial culture (Qiagen, UK).

PCR and characterisation for ESBL-PE and CRE encoding genes

PCR was performed using Rotor-Gene Q (RGQ) (Qiagen, UK) and positivity detected by the RGQ software. An in-house ESBL/carbapenemase high-resolution melt analysis assay which enables simultaneous detection of the 14 most important ESBL, carbapenemase and AmpC genes was used for gene characterisation.[23]

Data analysis

All personal identifiers were redacted, and samples marked with unique identifiers were linked to demographic data stored in a multiple factor authentication encrypted database. We performed a descriptive analysis of the overall baseline MDR-GNB prevalence rates and characterised the species identified.

RESULTS

Demographics

239/250 samples collected from all groups were suitable for analysis. Insufficient sample volume (3/250), duplicate samples (5/250), unlabelled samples (2/250) and illegible sample labelling (one) were reasons for exclusion from analysis. 204/239 (85.4%) of the samples were from male participants with a median age of 31 years (IQR 26–37) (Table 2).

AMR gene carriage and species identification

Overall, faecal samples of 40/239 (16.7%) personnel were found to contain ESBL genes. *E. coli* was detected in 39/40 (97.5%), while *K. pneumoniae* was found in 1 (2.5%) of the samples. No other organisms were detected on the selective plates. The most prevalent ESBL genotype overall was the SHV gene, in 33/40 (82.5%) of cases. A CTX-M gene was detected in 23 (57.5%) samples: 16/40 (40.0%) had CTX-M group 1 genes (CTX-M 1) and 8/40 (20.0%) had CTX-M group 9 genes (CTX-M 9) (one had both). The TEM gene was present in 18/40 (45.0%). One sample of *E. coli* carried all four of: CTX-M 1 and 9, SHV and TEM genes. The *K. pneumoniae* sample had an SHV-type gene alone. 18 of 40 (45.0%) carried a CTX-M gene and either an SHV or TEM gene (Figure 1).

The predeployment cohort (cohort 1) provided 157/239 (65.7%) of all samples. An ESBL-PE was identified in 29/157 (18.5%), all of which were *E. coli*. The most frequently observed ESBL gene was the SHV gene in 22/29 (75.8%), followed by CTX-M carriage in 18 (62.0%), including CTX-M genotype 1 in 11 (37.9%) and CTX-M 9 in 7 (24.1%). The TEM gene was present in 12 (41.3%). Carriage of a CTX-M gene and either SHV or TEM and was observed in 6/29 (20.6%).

Cohort 2 had an overall ESBL carriage rate of 8/63 (12.7%), with *E. coli* isolated from seven (87.5%) samples and *K. pneumoniae* from one. The SHV gene was found in all eight, and CTX-M 1 and TEM genes were each present as well in 4/63 (6.3%) samples. Three samples had all three genes (CTX-M 1, SHV and TEM).

ESBL carriage was found in 3/19 (15.8%) samples from cohort 3, all *E. coli*. SHV gene carriage was observed in all isolates, together with a TEM gene in two, and a CTX-M gene in one.

Table 2 Demographic comparisons between different cohorts.

Demographics	N	Median age (years) (IQR)	Male	Other ranks	SNCOs and officers
Cohort 1 (Pre deployment)	157	28 (23–37)	143/157 (91.1%)	141/157 (89.8%)	16/157 (10.2%)
Cohort 2 (During deployment)	63	24.5 (22–28.8)	49/63 (77.8%)	49/63 (77.8%)	14/63 (22.2%)
Cohort 3 (Post deployment)	19	35 (30–38)	19/19 (100%)	10/19 (52.6%)	9/19 (47.4%)

Ranks are defined using the North Atlantic Treaty Organisation rank range. Other ranks=OR1–OR4; senior non-commissioned officers (SNCOs)=OR5–OR9; officers=OF1–OF10

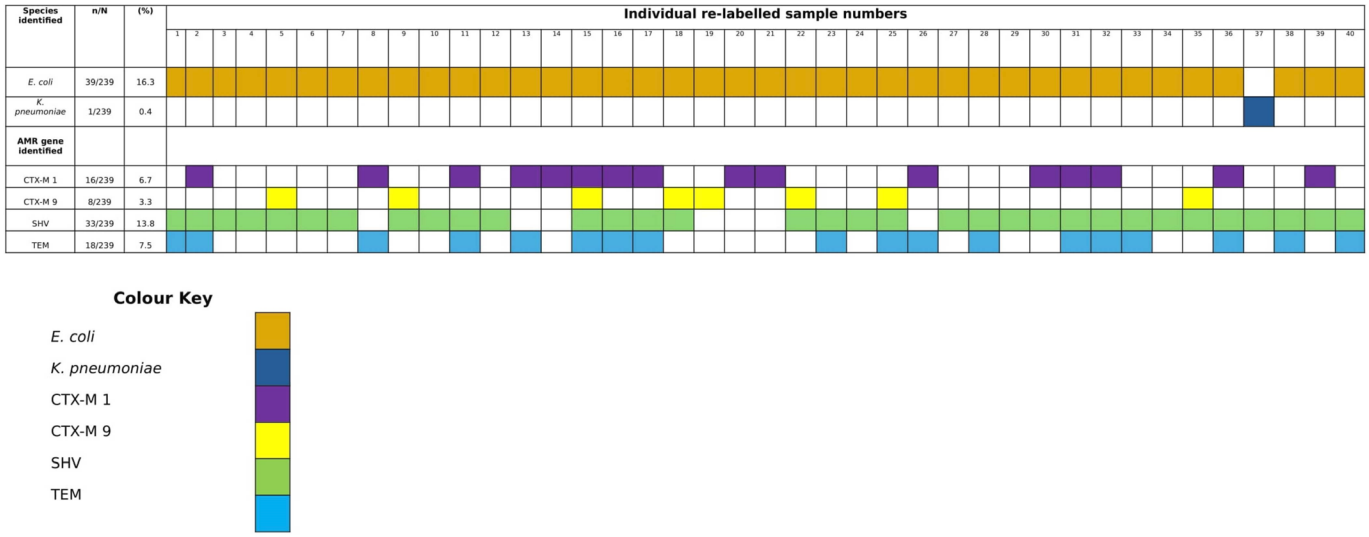

Figure 1 Distribution of species and antimicrobial resistance genes in faecal samples from 40 individuals with extended-spectrum β-lactamase-producing Enterobacterales. Only isolates positive for at least one AMR species are show. AMR, antimicrobial resistant; CTX-M 1, cefotaxime hydrolysing β-lactamase (Munich) 1; CTX-M 9, cefotaxime hydrolysing β-lactamase (Munich) 9; *E. Coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; SHV, sulfhydryl reagent variable type; TEM, Temoniera type.

DISCUSSION

In this study of nine different groups of British military personnel, the prevalence of ESBL carriage in faeces was 40/239 (16.7%), of which 39 (97.5%) were ESBL-producing *E. coli*. The most common ESBL genotype was the SHV gene, in 33/40 (82.5%); 18 (45.0%) samples contained both a CTX-M gene and either an SHV or TEM gene. No CRE were detected. This is the largest study of MDR-GNB carriage in British SP.

Previous data showed a prevalence of ESBL-producing *E. coli* in 11% of the general population of England, Wales and Scotland combined, but that report did not estimate total ESBL carriage rates and the surveillance was undertaken 10 years ago.[8] Our ESBL-PE prevalence is higher than the prevalence reported in England alone of 7.3%; however, UK-wide data on total carriage are scarce.[24] Acquired carbapenemase-producing organisms rates in England are relatively low,[14] and we detected none.

The demographics of the personnel who provided faecal samples is typical of a normal military deployment, with men in the lower ranks predominating. Deployments ranged from 4 weeks to 2 months and were generally to low-to-middle-income countries, which are linked to increased MDR-GNB exposure.[25] More encouragement is needed for general chain of command engagement to facilitate study uptake, but contrastingly, personnel who deployed to Kenya during the diarrhoeal outbreak we cited earlier were more responsive.

Our results differ from the higher rates found in a recent longitudinal study in Kenya, where ESBL-PE were found in the faeces of 26.5% of 121 individuals.[17] We found carriage in 29/157 (18.5%) in the predeployment cohort (including from 29 individuals whose samples were collected within 5 days of their arrival in Kenya), and in 8/63 (12.7%) in cohort 2 during their training in Kenya, which immediately preceded recruitment for the trial evaluating chemoprophylaxis against travelers' diarrhea (PREVENT-TD) report.[17] Similar methodology was used to detect ESBL-PE in both studies, and the reasons for the apparent differences are unclear. They may reflect reduction of travel during and immediately after the lifting of COVID-19 restrictions, which is when some of our samples were collected, whereas the PREVENT-TD study was conducted earlier. However, data supporting an association between travel restrictions and the curtailment of MDR-GNB spread are limited.

Colonisation and infection with all MDR-GNB, including ESBL-PE and CRE, are a significant threat to wounded military personnel, especially in the context of abdominal trauma.[21] In a US study involving 99 military travellers, ESBL-producing *E. coli* colonisation occurred in eight travellers (incidence density 3.5/1000 travel days; cumulative incidence 11% of trips (95% CI: 4% to 19%)). There was a higher rate of acquisition of ESBL-producing *E. coli* in association with travel to Asia compared with other regions (36% vs 7%, p=0.02).[26] Evidence of point source transmission within military populations remains scant, with one French study attributing ESBL acquisition by 72/189 (38%) personnel to external sources such as locally procured food and drink rather than direct contact.[19] In that study, ESBL-producing *E. coli* were found in 29/33 (88%) returning from Afghanistan and 39/80 (49%) from Côte d'Ivoire, compared with only 4/76 (5%) from French Guiana. Even higher rates of 70% were reported in Polish troops returning from a 6 month deployment in Afghanistan in 2019.[18] This emphasises the differing risks according to destination, and the need for surveillance to include regions with known higher prevalence of ESBL-PE, especially high-risk areas in the European, Eastern Mediterranean, Southeast Asian and Western Pacific WHO Regions.[27]

Globally, the CTX-M variant has replaced TEM and SHV in dominance.[28] Colonisation with CTX-M ESBL-PE in England was found in 7.3% of 2430 individuals a decade ago,[24] and it is the most recognised ESBL genotype out of three main variants (TEM, SHV and CTX-M).[29] Contrastingly, more SHV and TEM types than either CTX-M 1 or 9 groups were detected in this study. The combined proportion of CXT-M 1 and 9 in this study was 57.5% of 40 isolates, which is still relatively high and might be explained by regular overseas travel by our study population to some deployment regions of the world that may have high CTX-M rates.

The latest ESPAUR data suggest increased resistance to third generation cephalosporins in the faeces of up to 17.4% of the population in England.[9] The proportion of bloodstream *E. coli* isolates resistant infections to most first-line agents also exceeded

10%, with 41.4% of isolates reported as resistant to co-amoxiclav.[9] These findings have important implications for antimicrobial stewardship within the UK Defence Medical Services (DMS).

This study only assessed the genotypic carriage of MDR-GNB and screened for the five main carbapenemases, although there are other clinically relevant enzymes or enzyme families; phenotypic testing was not performed due to time and financial constraints. The next steps would be to perform antibiotic susceptibility testing (AST) to understand the phenotypic scope of ESBL and to provide further data to guide the review of the current DMS deployed antibiotic formulary. For example, current operational guidance for ballistic trauma recommends administration of intravenous co-amoxiclav 1.2 g three times daily as the first line antibiotic. Guidelines on alternative options should be discussed. More phenotypic data would need to be reviewed before a carbapenem is recommended as the empirical antibiotic of choice. The ESPAUR report further highlights the need for consideration of alternative antibiotics as empirical regimens in sepsis,[9] which is highly relevant in military trauma. However, patient management decisions are likely to be influenced by deployed settings and other military considerations.

SHV was detected in most of the of isolates in the predeployment cohort, and while the overall rates might match, the distribution of genotypes does not match UK-wide data. This emphasises the need for surveillance of MDR-GNB within the tri-service British military environment. We recommend commissioning a similar prospective 3-year iteration of this study, incorporating AST as routine to effectively monitor any changes in MDR-GNB carriage. We did not observe any CRE, which is comparable to current UK civilian data, but the DMS and broader British Armed Forces should remain vigilant for the spread of these significantly difficult to treat organisms.

There are several limitations to our study, including the heterogeneous cohorts recruited, and the small numbers in each group. As a result, detailed analysis of differences between groups could not be explored with adequate statistical power. Few personnel were recruited from some deployment destinations with known higher risk of ESBL carriage, and we did not obtain sequential samples within cohorts. We had initially planned to collect faecal samples before and after deployment to evaluate the dynamics of travel-related acquisition of ESBL and CRE, but for operational reasons some participants were deployed elsewhere at short notice or were only able to provide faecal samples at one time-point. Consequently, we were unable to match corresponding participant samples between any of the three cohorts to determine time-points at which MDR-GNB were likely to have been acquired, or indeed to establish a direct association between travel destination and acquisition. Given the potential heterogeneity of the gut microbiome and selection of a single colony in each isolate for analysis, duplicate samples in future studies could be included in the analysis. Most samples had been in prolonged cold storage prior to testing, which could lead to degradation of faecal DNA/RNA,[30] so the prevalence rates in this study could be under-estimates. Nevertheless, this moderate sized study of MDR-GNB carriage in diverse settings establishes a baseline carriage rate of MDR-GNB in British SP. This is useful for comparison with national civilian data, and with fellow coalition forces such as North Atlantic Treaty Organisation and Five-Eyes partnership nations. These data can support reviews of the DMS deployed antibiotic formulary instead of relying on UK national data.

CONCLUSIONS

Current MDR-GNB carriage in the British military is comparable to the overall UK national data, but SP may deploy to locations with high MDR-GNB carriage rates. Sustained surveillance programmes are needed to link MDR-GNB carriage and genotypic variation with travel destinations in this highly dynamic and mobile population. These data, combined with the identification of virulence features, have important antimicrobial stewardship and policy implications in terms of empirical first-line and second-line treatment decisions. This is vitally important in critical emergencies such as penetrating abdominal trauma and is of significant consideration within the wider austere deployment environment force health protection context.

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Contributors NJB, DSB, TF, MKO and TE advised on study design and concept. RT, SW, WN, TF and MKO completed the ethics application, and RT co-ordinated study logistics and administrative tasks. RT, CB, JK, MR, WN and IH recruited participants and collected faecal samples. RT, WN, IH, CB, TMR and TE processed samples. TMR conducted the DNA extraction and PCR testing for encoding genes. RT drafted the initial manuscript, and edits were made by SW and NJB. TF, MKO, NJB, SJCP and SW reviewed the manuscript. All authors contributed significantly to the final revisions and agreed on the version submitted to the journal and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. RT and SW are guarantors and accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. British military personnel who provided informed written consent as part of two studies of gastrointestinal infection were eligible to take part in the study. We had prior associated consent for future use of stored samples in one of the studies. Ethical approval was granted by the UK Ministry of Defence Research Ethics Committee (MODREC) (2047/MODREC/21 and 2076/MODREC/21) (both granted in 2021). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data that support the findings of this study are available on request from the corresponding author (RT; Romeo.Toriro@lstmed.ac.uk) on reasonable request, provided this meets local ethical and research governance criteria. All data are freely accessible.

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