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High prevalence of methicillin-resistant *Staphylococcus aureus, Giardia*, and *Blastocystis* in asymptomatic Syrian asylum seekers in Denmark during 2016 through 2018

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ABSTRACT

Introduction: Concerns have been raised regarding the emergence of antimicrobial-resistance and parasitic infections in the European refugee population. Here, we estimated the prevalence of intestinal parasites and selected antimicrobial-resistant bacteria in newly arrived asylum seekers in Denmark.

Materials and methods: Using a cross-sectional one-stage cluster sample design, adult Syrian asylum seekers were included upon arrival in Denmark. Faecal samples were collected and tested for ova and parasites, extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-E) and carbapenemase-producing organisms (CPO). Throat swabs were collected and analysed for methicillin-resistant *Staphylococcus aureus* (MRSA) and *Corynebacterium diphtheriae*.

Results: We invited 121 eligible individuals (20% of the source population) from six different asylum centres to participate, of whom 113 agreed. Throat swabs and faecal samples were received from 104 and 48 participants, respectively. Seven individuals did not provide enough material for the entire panel of faecal analyses. Three individuals (7.3%, 95%CI: 2.5–19.4%) were colonised with *Giardia intestinalis* and 28 (68.3%, 95%CI: 53.0–80.4%) with *Blastocystis* sp. (subtypes 1 [n = 5], 2 [n = 9] and 3 [n = 14]). Seven individuals (6.7%, 95%CI: 3.3–13.3%) were colonised with MRSA and one with ESBL-E. None had CPO or *Corynebacterium diphtheriae* and none reported any gastro-intestinal symptoms.

Discussion: Even with the most conservative estimates, the prevalence of *Giardia intestinalis, Blastocystis* sp. and MRSA was high in this asymptomatic refugee population.

Conclusions: We highlight the importance of raised awareness of antimicrobial-resistant bacteria when attending to newly arrived Syrian refugees. Meanwhile, our data suggest that routine screening for intestinal parasites in this population is of limited clinical relevance.

1. Introduction

By the end of 2018, the European Union (EU) hosted almost 2.5 million refugees of whom approximately 36,000 lived in Denmark (The World Bank, 2019, UNHCR, 2019, UNHCR popstats, 2019). In comparison, in 2014, the number of refugees in the EU and Denmark was about 1 million and 18,000, respectively. The large majority of refugees in the EU are from the Middle East, and a traditional, although largely unfounded, concern in public health is the spread of infectious diseases and antimicrobial-resistant bacteria to the autochthonous population in the host country (Abubakar et al., 2018, Castelli and Sulis, 2017). Symptoms of intestinal parasite colonisation or infection range from absent or mild, through gastrointestinal complaints to organ failure, depending on the agent in question. Recent studies have found a relatively high prevalence (ranging from 17% to 59%) of gastrointestinal parasites in refugee populations in Swe-

Abbreviations: ESBL-E, extended-spectrum beta-lactamase-producing Enterobacterales; CPO, Carbapenemase-producing organisms; MRSA, Methicillin-resistant *Staphylococcus aureus*; C. diph, *Corynebacterium diphtheriae*; MALDI-TOF, Matrix-assisted laser desorption-ionization time-of-flight; IQR, inter-quartile range; CI, confidence interval; ANOVA, Analysis-Of-Variance.

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Table 1
Overview of sample collection and analyses.

Material	Collection instrument	Storage	Shipment	Outcome of interest	Diagnostic methods
Faeces	Specimen containers with spoon, protective shipment container	Refrigerated, +5 °C	Regular postal service, in protective shipment container	Resistance pattern: ESBL & CPO	Culture
				Bacteria	MALDI-TOF
				Ova and parasites	Microscopy & Next-generation sequencing
Throat swab	BD ESwab(TM) collection kit regular flocked swab system			MRSA & *C. diphtheriae*	Culture

Abbreviations: ESBL-E, extended-spectrum beta-lactamase-producing *Enterobacterales*; CPO, Carbapenemase-producing organisms; MRSA, Methicillin-resistant *Staphylococcus aureus; C. diphtheriae* , *Corynebacterium diphtheriae*; MALDI-TOF, atrix-assisted laser desorption-ionization time-of-flight. The microbiological procedures are described in detail in (Eiset et al., 2020).

den, USA, and Canada (Benzeguir et al., 1999, DeVetten et al., 2017, Peterson et al., 2001) and abdominal pain—an unspecific symptom often linked to being colonised with gastrointestinal parasites—is consistently found to be one of the most common complaints among refugees seen in primary care or in the emergency department (Eiset and Wejse, 2017, Padovese et al., 2014, Sariaydin et al., 2018, Xu et al., 2018). Antimicrobial-resistant bacteria is a public health concern in general and in particular in migration health because of the risk of dissemination of the antimicrobial-resistant genes to otherwise susceptible organisms (accessed November 26, 2020, MacPherson et al., 2009). Previous studies of asylum seeker populations residing in EU countries have reported a high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) (Aro and Kantele, 2018, Fulchini et al., 2019, Heudorf et al., 2016, Ravensbergen et al., 2017) and extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales (Fulchini et al., 2019, Heudorf et al., 2016, Ravensbergen et al., 2017, Ehlkes et al., 2019, Angeletti et al., 2016), but very rarely reported on carbapenemase-producing organisms (CPO) (Fulchini et al., 2019, Ehlkes et al., 2019).

Many of these studies are limited by small sample sizes, suboptimal sampling frames and very heterogeneous study populations, exemplified by the grouping of refugees from markedly different countries of origin, with different migration history and different time spent in the host country. Finally, in 2015, three EU countries reported nine cases of cutaneous diphtheria in refugees (one in Denmark) seven of which were toxigenic (ECDC, 2015). This situation developed into an outbreak but drew the attention of public health practitioners to asylum seekers' living conditions as a risk factor for local outbreaks with a potentially high fatality risk. In this paper, we aimed to estimate the prevalence of colonisation with intestinal parasites and ESBL-producing *Enterobacterales,* CPO, MRSA, *Corynebacterium diphtheriae* (*C. diphtheriae*) in a Syrian-born asylum seeker population newly arrived in Denmark.

2. Methods

The study was part of the "Asylum seekers' and Refugees' Changing Health" (ARCH) project which is described in detail in the study protocol (Eiset et al., 2020). Briefly, adult Syrian asylum seekers were included shortly after arriving in Denmark during 2016 through 2018 using a cross-sectional design with one-stage cluster sampling, setting the design effect to one due to random allocation to the asylum centres [22]. The inclusion criteria were: (a) Syrian-born, (b) adult (>18 years old), (c) fled Syria after February 2011 (*i.e.*, after the onset of the current Syrian war), (d) arrived in Denmark less than 12 months prior to inclusion and (e) resident in a refugee centre at the time of inclusion. We excluded individuals physically and/or mentally incapable of participating. As part of the general clinical examination undertaken by health-care professionals, participants were asked for a throat swab and three faecal samples, and their medical history was recorded. Informed consent (both written and oral) was secured from each individual before enrolment. Participants were informed of their right to withdraw from the study at any time, to refuse to give information on any question and to submit one or more of the requested samples. The participants were thoroughly informed that their participation would have no consequences for the processing of their asylum application. The reporting conforms to the STROBE statement (Vandenbroucke et al., 2007); a filled-out checklist is provided in Supplementary Data 1. The R code for all analyses, including those in the appendices, is available from the first author's GitHub page: https://github.com/eiset/ARCH_Asylum-seekers-and-Refugees-Changing-Health

2.1. Background information

A 20-item questionnaire on four constructs were developed to collect information on socio-demographics, health status, health behaviour and migration history. The questionnaire was translated into Arabic and back-translated into English, and the final edition approved by the first author and a team of collaborators in Lebanon.

2.2. Throat swabs and faeces samples

Table 1 provides a summary of specimen collection, storage, shipment and analyses. For the throat swabs, we cultured material from both tonsils and any oral lesions on selective media for MRSA (chromID MRSA; Biomerieux, France) and *C. diphtheriae (Selenite plate, SSI Diagnostics, Denmark)*. For the faeces samples participants were provided with three labelled specimen containers and instructed to submit samples from three different defecations over the following five days. Screening for ova and parasites was based on both microscopy and amplicon-based next-generation sequencing as described elsewhere (Krogsgaard et al., 2018, Ring et al., 2017, Stensvold et al., 2020). The faeces samples were cultured by plating 10 μL of medium on a chromogenic culture media (chromID ESBL; Biomerieux, France) for detection of ESBL and 1 ul on SSI enteric medium (SSI diagnostics, Denmark) with a meropenem Neo-Sensitabs^{TM} (Rosco, Denmark) for detection of CPE (using a breakpoint of 27/27 mm) and incubated aerobically at 36 °C. The plates were examined after 18–24 h of incubation. Confirmation of ESBL production was performed by the ESBL + AmpC Screen Kit (Rosco, Denmark). Confirmation of CPE was performed by the ROSCO Rapid CARB Screen kit (Rosco, Denmark) according to EUCAST standards (The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters using a breakpoint of 0.25/0.25 ug/ml. Identification of bacteria was done by use of Matrix assisted laser desorption-ionization (MALDI-TOF; Bruker, Germany) .

2.3. Statistics

Prevalence estimates were calculated with 95% confidence intervals (CI). We aimed to include 220 individuals to ensure that the CI

Table 2
Demographics and selected clinical data of the study population.

	Total study population($N = 113$)	One or more parasites detected($N = 30$)	No parasites detected($N = 11$)
Sex			
Female	53 (46.9%)	12 (40.0%)	5 (45.5%)
Male	60 (53.1%)	18 (60%)	6 (54.5%)
Missing	0	0	0
Age (years old)	30 (25.0–37.0)	33 (25.0–37.5)	32 (31.2–38.8)
Missing	10 (8.8%)	3 (10%)	1 (9.1%)
Time in Denmark (months)	8.5 (5.0–11.0)	8 (6–11.2)	12 (9.5–12)
Missing	5 (4.4%)	2 (6.7%)	0
Area of origin in Syria			
Aleppo	27 (38%)	7 (23.3%)	3 (27.3%)
Damascus	13 (18.3%)	1 (3.3%)	2 (18.2%)
Al-Hasakah	10 (14.1%)	4 (13.3%)	1 (9.1%)
Tartus	5 (7.0%)	1 (3.3%)	1 (9.1%)
Other	16 (22.5%)	3 (10.0%)	2 (18.2%)
Missing	42 (37.2%)	14 (46.7%)	2 (18.2%)
Followed child vaccination programme			
Yes	86 (80.4%)	23 (76.7%)	10 (91.0%)
No	9 (8.4%)	2 (6.7%)	0
Do not know	12 (11.2%)	4 (13.3%)	1 (9.1%)
Missing	6 (5.3%)	1 (3.3%)	0
Diagnosed with one or more diseases*			
Yes	33 (41.2%)	9 (30.0%)	6 (54.5%)
No	47 (58.8%)	10 (33.3%)	4 (36.4%)
Missing	33 (29.2%)	11 (36.7%)	1 (9.1%)

Categorical variables are represented as n (%), while continuous variables are represented as median (interquartile range).
* For simplicity, we do not report all diseases registered. No participant reported gastrointestinal related diseases or symptoms.

width would be no wider than ±7 percentage points, no matter the observed proportion. In short, we estimated the prevalence of colonisation and antimicrobial-resistance and its corresponding Wilson 95% CI (Agresti and Coull, 1998, Dean and Pagano, 2015). Finally, to qualify the discussion of the internal and external validity of our results, we performed missing data analysis by plotting the fraction of individuals with missing data on key variables, performing hierarchical cluster analysis and logistic regression and Analysis-Of-Variance (ANOVA) to identify the conditional missingness of data on outcomes. Data was entered and stored in the REDCap database (Harris et al., 2009) hosted at the first authors' research institution. All analyses and plots were made using the R software (R Core Team 2020).

The study was planned, conducted and reported in accordance with the *Recommendation for the Conduct, Reporting, Editing and Publication of Scholarly work in Medical Journals* (http://www.icmje.org/recommendations/) and approved by the Danish Data Protection Agency (file number 2015-41-4500). According to Danish law, no further permission was required for this type of study.

3. Results

Of an estimated source population of 613, we reached and invited a random sample of 121 eligible individuals from six different asylum centres, of whom 113 agreed to participate, resulting in a participation rate of 93.4% ($n = 113/121$). From these, a throat swab and at least one faecal sample were obtained and analysed from 92% ($n = 104/113$,) and 43% ($n = 48/113$) participants, respectively. Of the 48 individuals that delivered faecal samples seven delivered too little material to include the parasite analysis. The majority were relatively young (between 25 and 37 years old), from the two major Syrian cities (Aleppo and Damascus), had been in Denmark for eight and a half months prior to inclusion in the study, and had followed the vaccination programme in Syria which has good coverage of the most important vaccine-preventable diseases (WHO, 2020). Fig. 1 provides a graphical overview of the inclusion process and details on reasons for refusing to participate, and Table 2 provides an overview of the demographics of the study population and subpopulations according to parasite colonisation status.

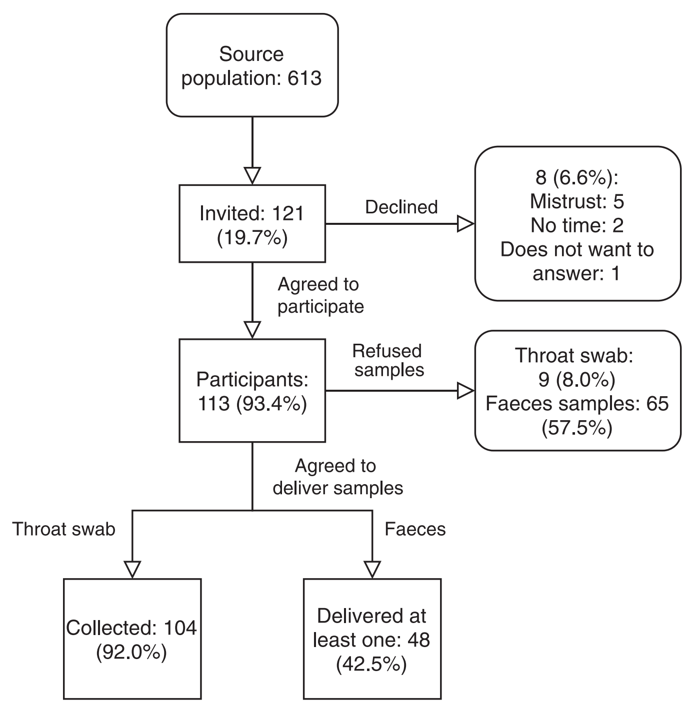

Fig. 1. Flow-chart of inclusion, reason for non-participation and number of delivered samples

Thirty individuals tested positive for at least one intestinal parasite, corresponding to a parasite detection rate of 73% ($n = 30/41$), and eight individuals had antimicrobial-resistant bacteria in the throat or intestine (seven had MRSA only, one had ESBL only; *i.e.*, none had both). Table 3 provides detailed information on the laboratory results. *Blastocystis* sp. was the parasite most commonly found: of the 28 *Blastocystis* sp.-positive individuals, 18 were co-colonised with one ($n = 9$), two ($n = 7$) or three ($n = 2$) other organisms (Supplementary Data 2). All three cases of *Giardia intestinalis* were co-colonised with *Blastocystis,* and two cases were

Table 3
Microbiology test results

	n	Prevalence in % (95%CI)
Throat swabs (*N* = 104)		
MRSA	7	6.7 (3.3; 13.3)
Corynebacterium diphtheriae	0	NA
Faeces - Bacteria and antimicrobial-resistance (*N* = 48)		
Escherichia coli	6	12.5 (5.9; 24.7)
ESBL-producing *Escherichia coli*	1	2.1 (0.1; 10.9)
CPO	0	NA
Faeces - Parasites (*N* = 41)		
Blastocystis spp.	28	68.3 (53.0; 80.4)
Blastocystis subtype 1	5	12.2 (5.3; 25.5)
Blastocystis subtype 2	9	22.0 (12.0; 36.7)
Blastocystis subtype 3	14	34.1 (21.6; 49.5)
Dientamoeba fragilis	9	22.0 (12.0; 36.7)
Entamoeba dispar	1	2.4 (1.3; 12.6)
Entamoeba coli	6	14.6 (6.9; 28.4)
Endolimax nana	3	7.3 (2.5; 19.4)
Entamoeba hartmanni	3	7.3 (2.5; 19.4)
Giardia duodenalis	3	7.3 (2.5; 19.4)
Iodamoeba bütschlii	1	2.4 (1.3; 12.6)

Abbreviations: ESBL, Extended-spectrum beta-lactamase; CPO, Carbapenemase-producing organisms; MRSA, Methicillin-resistant *Staphylococcus aureus*; NA, not applicable.

also positive for MRSA. The median age of the subpopulation with one or more intestinal parasites was 33 years (IQR, 25.0–37.5); in the subpopulation with no intestinal parasites detected it was 32 years (IQR: 31.2–38.8). The average length of stay in Denmark at the time of testing was shorter in the subpopulation colonised with one or more intestinal parasites or ESBL (both median of eight months) compared with the subpopulation not colonised with parasites nor with ESBL (both median of 12 months), whereas the opposite was true for the subpopulation colonised with MRSA (median of of 11 months) compared with the subpopulation not colonised with MRSA (median of eight months). None of the participants who delivered faecal samples reported any gastrointestinal symptoms such as abdominal pain, weight loss, diarrhoea or constipation, nor any current or prior treatment for gastrointestinal issues, except for one participant, who reported regular use of loperamide (antidiarrhoeal drug) and who tested negative for all outcomes; the individual could not remember the reason for initiation of this drug. Missing data analysis showed that refusal to deliver throat swab or faeces samples were independent of all covariates (see Supplementary Data 3–7 for plots and a detailed discussion).

4. Discussion

We found a high prevalence of parasites that have been associated with abdominal pain, anorexia, diarrhoea and weight loss (in particular *Blastocystis* sp. was common); and also a high prevalence of so-called non-pathogenic intestinal amoebae (such that *Entamoeba coli, Endolimax nana, Entamoeba hartmanni*). The 95% CI for the prevalence estimate for *Giardia intestinalis* colonisation ranged from 2.5% to 19.4%, which is noticeably higher than the prevalence estimate for the general population of 1% reported in a study from Finland (Kyrönseppä, 1993) and Denmark <1% in Denmark (Krogsgaard et al., 2018) (Supplementary). These results suggest that many participants had been exposed to faecal-oral contamination, however, none of the colonised individuals reported gastrointestinal complaints. In general, intestinal parasite infection is a rare cause of hospitalisation (Dam Larsen et al., 2017) and our study supports the hypothesis that in most cases, symptoms related to these parasites may be absent or mild. In accordance with previous studies, we found a high MRSA prevalence with 95% CI ranging from 3% to 13% and no individuals with CPO (Aro and Kantele, 2018, Fulchini et al., 2019, Heudorf et al., 2016, Ravensbergen et al., 2017, Ehlkes et al., 2019); however, contrary to these studies, we found a low prevalence of ESBL (one individual). With no cases of *C. diphtheriae* detected, we reaffirmed previous studies finding a very low prevalence of *C. diphtheriae* among Syrian refugees (Meinel et al., 2016) which is also in accordance with a reported high immunisation coverage (WHO, 2020).

Due to practicalities, such as collecting specimens in asylum centres and refugee camps, we were limited to analysing only one swab site (the throat) and did not include multiple swab sites even though this would have increased the sensitivity of MRSA tests (Ahmad et al., 2019, El-Bouri and El-Bouri, 2013). During participant inclusion, new policies were put in place in Denmark and the EU, leading to a remarkable drop in the number of Syrian asylum seekers arriving in Denmark. Thus, we did not reach our aim of including 220 participants. Moreover, less than half of the participants submitted stool samples for testing, and a large proportion of the participants did not deliver the instructed amount of faeces or number of samples. Together, this is reflected in uncertainty and instability about the prevalence estimates in question: for example, 48 individuals were tested for ESBL *Escherichia coli* of which one were positive resulting in a 95% CI ranging from negligible (0.11%) to quite common (10.90%), and a prevalence estimate that would double with just one more positive individual. For other agents, the point estimate may be imprecise, but the limits of the 95% CI is noteworthy nonetheless: for example in the case of MRSA, the lower bound of the 95% CI was 3% which corresponds to the reported MRSA prevalence in studies of nursing home residents in Europe (Aschbacher et al., 2016, Latour et al., 2019). We secured a random sample with participation rate of 93% that constituted almost 1/5 of the entire source population. Although the absolute numbers are low, this participation rate is among the highest for studies in difficult-to-reach populations (Ahlmark et al., 2014, Enticott et al., 2017, Nesterko et al., 2019, Tinghög et al., 2017). In Table 2 we provide demographic data for the study population and subpopulations stratified on colonisation status. With a difference of 4 months on average since arrival in Denmark this gives an indication that the burden of parasitic colonisation may be highest in the very recently arrived refugees, however further studies would have to investigate this more thoroughly. The missing data analysis, followed by discussions of possible missingness mechanisms among the authors, did not identify any predictors of non-acceptance to provide throat swabs or stool samples among the other variables measured, supporting that non-acceptance was not systematic and thus did not introduce data bias. This suggests that our results can be generalised to an adult population of Syrian asylum seekers hosted in European countries. Finally, our findings indicate that policy recommendations are warranted; we find there is a considerable yield to gain from adding screening for gastrointestinal pathogens and MRSA in refugee health assessments, which are currently conducted with vast differences according to varying local policies (Seedat, 2018).

5. Conclusions

This study showed a high prevalence of intestinal parasites and MRSA. Our results indicate that routine screening for intestinal parasites in asymptomatic adult Syrian refugees is not justified: although the majority tested positive for one or more intestinal parasites, these individuals reported no symptoms. MRSA is a public health threat, particularly if disseminated in vulnerable populations such as in hospitals. Thus, we emphasise the importance of a raised awareness of antimicrobial-resistant bacteria when attending to Syrian refugees in particular when hospitalised; furthermore, it may be reasonable to incorporate screening for MRSA and gastrointestinal pathogens in refugee health assessments.

Author contributions

AHE conceived the study, made the analysis plan, wrote the initial draft of the manuscript and is the chief investigator on the ARCH research project and the principal investigator in Denmark. KF, HVN and CRS planned and carried out the laboratory analysis and discussed the results with AHE. CW advised in the initial phase of the project. All authors contributed to and approved the final manuscript.

Declaration of Competing Interest

None.

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Supplementary materials

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