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Risk factors for household colonization by extended-spectrum cephalosporin-resistant enterobacterales (ESCrE) in Botswana

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Abstract

Background The epidemiology of community colonization with extended-spectrum cephalosporin-resistant Enterobacterales (ESCrE) in low- and middle-income countries (LMICs) is largely uncharacterized. In the community, the household is of particular importance. Identifying risk factors for household ESCrE colonization is critical to inform antibiotic resistance reduction strategies.

Methods Participants were enrolled at 6 clinics in Botswana. All participants had rectal swabs collected for selective plating and confirmation of ESCrE. Data were collected on demographics, comorbidities, antibiotic use, healthcare exposures, travel, and farm/animal contact. Households were considered exposed if any member had the exposure of interest. Households with ESCrE colonization (cases) were compared to non-colonized households (controls) to identify risk factors for household ESCrE colonization.

Results From 1/1/20 – 9/4/20, 327 households were enrolled. The median (IQR) number of people enrolled per household was 3 (2–4) ranging from 2 to 10. The median (IQR) age of subjects was 18 years (5–34) and 304 (93%) households included at least one child. Of 327 households, 176 (54%) had at least one household member colonized with ESCrE. Independent risk factors [adj OR (95%CI)] for household colonization were: (1) horse/donkey exposure [2.32 (1.05, 5.10)]; (2) yogurt consumption [1.73 (1.04, 2.88)]; (3) region [2.83 (1.48,5.43)]; and (4) enrollment during pre-COVID lockdown [2.90 (1.66, 5.05)].

Key points

• ESCrE colonization is common in households in Botswana and varies by geographic region and time period.

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Conclusions ESCrE household colonization was common with evidence of geographic variability as well as a possible role of animal exposure. The role of yogurt exposure requires further study with consideration of source (commercial, homemade). Further prospective studies of household ESCrE colonization with longitudinal assessments of exposures are required to identify effective prevention strategies.

Keywords Antibiotic, Resistance, Colonization, Extended- spectrum cephalosporin, Low and middle income countries, Household

Background

Infections due to multi-drug resistant gram-negative bacteria have become a global public health concern, due to high mortality rates and few effective treatments [1, 2, 3]. Specifically, extended-spectrum cephalosporin-resistant Enterobacterales (ESCrE) are classified by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) as serious/critical threats [4, 5]. Elucidating the epidemiology of ESCrE colonization is critical because colonization is a strong predictor of subsequent ESCrE infection and because individuals colonized with ESCrE may serve as reservoirs for transmission [6, 7, 8, 9]. The emergence of these organisms has been primarily studied in hospital settings, but studies addressing risk factors for acquisition and transmission within the community are few [10, 11]. Given the increasing global prevalence of ESCrE in community settings, studying its epidemiology is critical if efforts to curtail further emergence of ESCrE are to be successful [12, 13]. Low and middle income countries (LMICs) have often reported higher rates of community ESCrE colonization and lack surveillance infrastructure to track the spread of antibiotic resistant organisms [14, 15, 16, 17, 18, 19]. Despite this, the vast majority of studies that have investigated community transmission dynamics have been conducted in high income countries [6, 10, 20, 21, 22]. As such, there is an urgent need to better define the epidemiology of community ESCrE colonization in LMICs. In the community, the household is likely of particular importance as it is the setting in which individuals spend a majority of their time and may serve as a site of potential transmission. In this study, we analyzed households from three regions in Botswana and determined risk factors for colonization on a household level.

Methods

This research was conducted as part of the Antibiotic Resistance in Communities and Hospitals (ARCH) collaborative assessing antibiotic resistance in numerous global settings. This study was reviewed and approved by the institutional review boards of the University of Pennsylvania, University of Botswana, and the Botswana Ministry of Health and Wellness.

Study sites

As described previously [23, 24], this study was conducted as part of a surveillance project in the capital city and two villages across three districts in Botswana: (1) Gaborone; (2) Mochudi; and (3) Molepolole. Participants were enrolled from six outpatient clinics total, two from each region.

Study participants

Participants were enrolled from January 15, 2020, to September 4, 2020, with a pause during April 2, 2020, through May 21, 2020, due to a country-wide COVID-19 lockdown. Patients (> 18 years old) at a participating clinic were randomly selected for the study from among all patients present at the clinic at that time. If a participant provided informed consent, they were interviewed and underwent rectal swabbing by a trained research nurse. The next participant was then selected at random from among the clinic patients present.

Each enrolled clinic patient was asked to refer, if possible, three additional adults to the study. Referrals were encouraged to be from both within and outside the enrolled participant's household. In addition, all adults were asked to refer their children for participation. All referrals were enrolled in the same manner as the clinic patients. Community participants were enrolled within two weeks of the referring clinic patient's enrollment. Each participant could be enrolled only once.

Since the goal of this project was to evaluate household colonization with ESCrE, only those participants who referred at least one other household member were included. In this way, each household enrolled in the current study had at least two people from the household enrolled (i.e., there were no single person households). An index participant was defined as the first individual to be enrolled in the household.

Microbiological evaluation

All rectal swab samples were collected using FecalSwabs (Copan Italia, Brescia, Italy) according to the previously described workflow [24, 25]. Swabs were inoculated onto chromogenic media (CHROMagar™ ESBL) for preliminary identification of ESCrE. Further identification and phenotypic susceptibility testing was conducted using the VITEK MS matrix-assisted laser desorption/ionization-time of flight mass spectrometry system (bioMerieux,

Durham, USA) and VITEK-2 with the AST-GN89 card (bioMerieux, Durham, USA), respectively. ESCrE were defined as Enterobacterales demonstrating nonsusceptibility to ceftriaxone or ceftazidime [26]. This definition was designed as a phenotypic definition of likely ESBL-producing Enterobacterales (ESBL-EB).

Data collection

Clinical data were collected by the research nurse during each participant's enrollment. Each participant from a new household was given a household ID to track subsequent participants who identified as being part of the same household. We asked about demographics, comorbidities, exposures to the healthcare system (specifically hospitals and clinics) in the last six months, travel within the past six months, and antibiotic use in the last six months. Further information was collected on activities such as tending of crops and animals; handling and preparation of fish, chicken, and meat; and handling, treating, or disposing of waste from outside the household. In addition, we asked about main sources of drinking water and water for other purposes, along with sanitation facilities. We also inquired about general animal exposure and specific food consumption (e.g., meat, eggs, yogurt), along with the frequency of these behaviors. Lastly, we assessed household characteristics including number and ages of people in the household and how many other members of the household were also enrolled in the study.

Data analysis

The goal of this project was to compare households in which there was at least one person colonized with ESCrE (case households) to those households in which no one was ESCrE colonized (controls). While various data elements were ascertained from individual household members, categorization of these variables was conducted at the household level. For example, while we assessed recent travel in all household enrollees, we categorized travel as having been present in the household if any household member reported a positive travel history.

In unadjusted comparisons of case and control households, continuous variables were compared using the Student's *t*-test or Wilcoxon rank-sum test, and categorical variables were compared using the χ^2 or Fisher exact test. For the adjusted analyses, multivariable logistic regression was employed. Variables from bivariable analyses with *p* values < 0.20 were considered for inclusion in the final multivariable model. Variables were forced into the model based on biologic plausibility and collinear variables were excluded. Collinearity was investigated by assessing the variance inflation factor level. We evaluated whether a statistical interaction was present between the exposures and outcome of interest based on the time period (i.e., pre- vs. post-lockdown) as well as based on the geographic region (i.e., Gaborone, Mochudi, Molepolole).

Variables were retained in the final model if they had a *p* value of < 0.05 in the multivariable model or based on a priori hypothesis. The strength of the association was measured using an odds ratio (OR). A 95% confidence interval (CI) was also calculated for each effect estimate. All analyses were performed using STATA v.18 (StataCorp, 2021) or R (R Core Team, 2021).

A secondary analysis included only those households in which at least one member was colonized with ESCrE. Within this subset, we compared households with more than one person colonized (i.e., case households) to households with only one person colonized (i.e., control households). The approach to unadjusted and adjusted analyses described above was also employed for this secondary analysis.

Results

During the study period, 1101 individuals were enrolled. This was comprised of 327 index participants and thus 327 households (Table 1). Among all households, 109 (33.3%) were enrolled in Gaborone, 131 (40.1%) were enrolled in Mochudi, and 87 (26.6%) were enrolled in Molepolole.

The median (interquartile range; IQR) number of people enrolled per household was 3 [2, 3, 4], ranging from 2 to 10. The median age (IQR) of index participants was 34 (27–43) and 290 (88.7%) were female. The median (IQR) age of individuals enrolled in households was 18 (5–34) and 304 (92.9%) households included at least one child. The median proportion of people available in a household that were enrolled was 0.71 (0.5-1.0).

Of 327 households, 176 (53.82%) had at least one household member colonized with ESCrE.

The index participant was colonized in 98 of 176 households (55.68%); in 78 (44.32%), the index participant was not colonized but at least one other household member was colonized. Of a total of 345 ESCrE isolated, 280 (81.2%) were *Escherichia coli*, 51 (14.8%) were *Klebsiella pneumoniae*, 8 (2.3%) were Citrobacter species, 5 (1.5%) were Enterobacter species, and 1 was *E. fergusonii*. Among all 345 ESCrE, the percent susceptible to various antibiotics were: meropenem (99.7%), amikacin (99.7%), ertapenem (98.6%), piperacillin (90.7%), tobramycin (88.4%), gentamicin (85.5%), nitrofurantoin (83.2%), ciprofloxacin (61.5%), cefepime (50.1%), and tetracycline (29.6%).

Among the 176 households, 103 (31.5%) had one person colonized, 41 (12.6%) had two people colonized, 17 (5.2%) had three people colonized, 11 (3.4%) had four people colonized, and 4 (1.2%) had five people colonized. Among households in which there were multiple ESCrE

Table 1 Bivariable analyses: risk factors for household ESCrE Colonization– Demographics, comorbidities, travel, antibiotic exposure, and healthcare exposures

Variable	Cases (ESCrE+) (*n* = 176) *N* (%)	Controls (ESCrE-) (*n* = 151) *N* (%)	OR (95%CI)	*P* value
General				
Median (IQR) age of people in household	19.5 (5–34)	17 (5–34)	--	0.99
# (%) households enrolled in the pre-lockdown period	95 (54.0)	39 (25.8)	3.36 (2.05–5.55)	< 0.001
Region # (%) Gaborone # (%) Mochudi # (%) Molepolole	72 (40.9) 67 (38.1) 37 (21.0)	37 (24.5) 64 (42.4) 50 (33.1)	---	0.003
Travel outside the country in the past 6 months	8 (4.55)	6 (3.97)	1.15 (0.34–4.12)	> 0.99
Comorbidities				
Diabetes mellitus	5 (2.84)	4 (2.65)	1.07 (0.23–5.52)	> 0.99
Respiratory disease	3 (1.7)	7 (4.64)	0.36 (0.06–1.6)	0.2
HIV	79 (44.89)	67 (44.37)	1.02 (0.64–1.62)	> 0.99
Hypertension	30 (17.05)	32 (21.19)	0.76 (0.42–1.38)	0.4
Antibiotic use				
Received at least one antibiotic in past 3 months	30 (17.05)	21 (13.91)	1.27 (0.67–2.46)	0.45
Healthcare Exposures				
Visited hospital to receive care	69 (39.2)	45 (29.8)	1.52 (0.93–2.48)	0.08
Visited clinic to receive care	161 (91.48)	141 (93.38)	0.76 (0.30–1.88)	0.54
Visited hospital for reasons other than receiving care	42 (23.86)	27 (17.88)	1.44 (0.81–2.58)	0.22
Visited clinic for reasons other than receiving care	97 (55.11)	88 (58.28)	0.88 (0.55–1.40)	0.58
Visited hospital for any reason	83 (47.2)	57 (37.7)	1.47 (0.92–2.35)	0.10
Visited clinic for any reason	165 (93.8)	148 (98.0)	0.31 (0.05–1.18)	0.097

Notes:

Healthcare exposures assessed in six months prior to enrollment

Variables reported only if present in at least 10 households or $p \leq 0.20$, or associations of interest

Exposure considered present if noted in at least one household member

isolates identified, the proportion of households for which the same species of organism was found from multiple isolates from within the household was 87.4%.

When comparing the pre-lockdown and post-lockdown periods, the median (IQR) number of people enrolled per household (regardless of colonization status) was 3 [2, 3, 4] in both periods. Of the households enrolled pre-lockdown and post-lockdown in which at least one person was ESCrE-colonized, the median (IQR) proportion of people ESCrE- positive in the household was 0.5 (0.33–0.67) and 0.5 (0.33–0.57), respectively. Finally, of the households enrolled pre-lockdown and post-lockdown in which at least one person was ESCrE-colonized, the median (IQR) number of people ESCrE- positive in the household was 1 (1–2) and 1 (1–2), respectively.

In comparing case and control households, antibiotic use and recent travel among household members were not significant risk factors for ESCrE colonization (Table 1). When looking at healthcare exposure, case households were more likely to have at least one individual who visited a hospital for care and more likely to have visited a hospital for any reason (Table 1). In terms of animal exposure, case households were significantly more likely to have interacted with livestock and horses/donkeys in the past week (Table 2). In terms of food consumption, yogurt consumption was significantly more common in case households (Table 2). Goat milk was of borderline significance. When looking at sanitation facilities, case households were significantly more likely to have a pit latrine without a slab than to have a flush to septic tank facility (Table 3). Cases were also more likely to not have soap for handwashing (Table 3). The median (IQR) number of people enrolled per household was 4 [3, 4, 5] in both case and control households.

In stratified analyses, there was no effect modification by time period (i.e., pre- vs. post-lockdown) or geographic region (i.e., Gaborone, Mochudi, Molepolole).

In multivariable analyses, we found the following variables to be significant risk factors for household ESCrE colonization: horse/donkey exposure [adj OR (95%CI) = 2.25 (1.03–4.91); $p = 0.04$]; hospital exposure [adj OR (95%CI) = 1.73 (1.05–2.85; $p = 0.03$]; use of pit latrine without slab/open pit [adj OR (95%CI) = 4.13 (1.53–11.11; $p = 0.005$]; yogurt consumption [adj OR (95%CI) = 1.90 (1.15–3.12); $p = 0.011$]; and geographic region (Gaborone) [adj OR (95%CI) = 2.66 (1.41–4.99); $p = 0.002$]. However, when controlling for time period (i.e., pre- vs. post-lockdown), both hospital exposure

Table 2 Risk factors for household ESCrE Colonization– Animal exposures and food consumption

Variable	Cases (ESCrE+) (*n* = 176); *N* (%)	Controls (ESCrE-) (*n* = 151); *N* (%)	OR (95%CI)	*P* value
Animal Exposures				
Interacted with livestock	72 (40.91)	45 (29.8)	1.63 (1.00–2.66)	0.04
Interacted with chickens	99 (56.25)	77 (50.99)	1.23 (0.78–1.96)	0.37
Interacted with turkeys or ducks	9 (5.11)	5 (3.31)	1.57 (0.46–6.11)	0.59
Interacted with horses or donkeys	30 (17.05)	12 (7.95)	2.37 (1.13–5.31)	0.02
Interacted with swine	2 (1.14)	1 (0.66)	1.72 (0.09–102.33)	> 0.99
Prepares fish chicken or other meats for consumption / sale	135 (76.7)	111 (73.51)	1.19 (0.69–2.02)	0.52
Food Consumption				
Meat	163 (92.61)	135 (89.4)	1.48 (0.64–3.48)	0.33
Poultry	161 (91.48)	138 (91.39)	1.01 (0.43–2.37)	> 0.99
Fish	68 (38.64)	50 (33.11)	1.27 (0.79–2.06)	0.36
Egg	113 (64.2)	90 (59.6)	1.21 (0.76–1.95)	0.42
Cow milk	156 (88.64)	137 (90.73)	0.80 (0.36–1.73)	0.59
Goat milk	8 (4.55)	16 (10.6)	0.40 (0.14–1.03)	0.054
Yogurt	131 (74.43)	88 (58.28)	2.08 (1.27–3.42)	0.002
Cheese	33 (18.75)	20 (13.25)	1.51 (0.80–2.92)	0.23
Fresh Vegetables	170 (96.59)	149 (98.68)	0.38 (0.04–2.17)	0.29
Fresh Fruit	164 (93.18)	139 (92.05)	1.18 (0.47–2.97)	0.83

Notes:

Exposure considered present if noted in at least one household member

All exposures assessed for the prior week

Table 3 Risk factors for household ESCrE Colonization - Water source, sanitation facilities, handwashing facilities, and waste handling

Variable	Cases (ESCrE+) (*n* = 176) *N* (%)	Controls (ESCrE-) (*n* = 151) *N* (%)	OR (95%CI)	*P* value
Main Drinking Water Source				
Piped water into dwelling	55 (31.25)	55 (36.42)	0.79 (0.49–1.29)	0.35
Piped water into yard/plot	158 (89.77)	143 (94.7)	0.49 (0.18–1.23)	0.11
Public tap/standpipe	6 (3.41)	4 (2.65)	1.30 (0.30–6.37)	0.76
Storage tank	10 (5.68)	5 (3.31)	1.76 (0.53–6.70	0.43
Sanitation Facilities				
Flush or pour-flush to piped sewage system	67 (38.07)	55 (36.42)	1.07 (0.67–1.73)	0.82
Flush or pour-flush to septic tank	14 (7.95)	23 (15.23)	0.48 (0.22–1.02)	0.053
Flush or pour-flush to pit latrine	8 (4.55)	13 (8.61)	0.51 (0.18–1.36)	0.17
Pit latrine with slab	134 (76.14)	117 (77.48)	0.93 (0.53–1.60)	0.79
Pit Latrine without slab/ open pit	24 (13.64)	6 (3.97)	3.8 (1.46–11.7)	0.003
No facilities or bush or field	5 (2.84)	6 (3.97)	0.71 (0.17–2.85)	0.76
Other toilet	50 (28.41)	37 (24.5)	1.22 (0.72–2.07)	0.45
Hand Washing				
Water	92 (52.27)	86 (56.95)	0.83 (0.52–1.31)	0.44
Soap	83 (47.16)	84 (55.63)	0.71 (0.45–1.13)	0.15
Reusable towel	10 (5.68)	10 (6.62)	0.85 (0.31–2.35)	0.82
Waste Handling				
Handle, treat or dispose of waste other than from household	72 (40.91)	64 (42.38)	0.94 (0.59–1.50)	0.82

Notes:

Variables reported only if present in at least 10 households or $p \leq 0.20$

and pit latrine use did not retain significance in the final model (Table 4). However, time period remained a significant risk factor for ESCrE household colonization in the final model (i.e., household colonization greater pre-lockdown). Of note, there was no significant interaction among variables in the final model.

In secondary analysis focused on only those households in which at least one member was colonized with

Table 4 Multivariable analysis of risk factors for household ESCrE colonization

Variable	Unadjusted OR	Adjusted OR (95%CI)	*P* value
Horse/Donkey Exposure [1]	2.37	2.32 (1.05–5.10)	0.037
Hospital Exposure [2]	1.47	1.30 (0.77–2.21)	0.327
Pit Latrine without Slab/Open Pit	3.80	2.06 (0.72–5.88)	0.177
Yogurt consumption [3]	2.08	1.73 (1.04–2.88)	0.036
Region (Gaborone)	2.63	2.83 (1.48–5.43)	0.002
Pre-Lockdown Time Period	3.37	2.90 (1.66–5.05)	< 0.001

[1] at least one person in the household with horse and/or donkey exposure in the past week

[2] at least one person in household with hospital exposure for any reason (i.e., care, visiting) in past 3 months

[3] at least one person in household with yogurt consumption in past week

ESCrE, we compared households with more than one person colonized (i.e., case households) to households with only one person colonized (i.e., control households). Among 176 households in which at least one individual was colonized with ESCrE, 103 (59%) households had only one person colonized and 73 (41%) had multiple people colonized. In multivariable analyses, the following variables were significantly associated [adj OR (95%CI); *p*-value] with multi-person household colonization: (1) time period (i.e., post-lockdown): [2.84 (1.42–5.64); $p = 0.003$]; and (2) median household age: [0.95 (0.92–0.98); $p = 0.002$]. Of note, the proportion of households with at least one enrolled person < 18 years of age was 86.3% and 70.0% for multi-person and single person colonized households, respectively [OR (95%CI) = 2.7 (1.17–6.68); $p = 0.01$]. In addition, the proportion of households with at least one person < 5 years of age was 69.9% and 48.5% for multi-person and single person colonized households, respectively [OR (95%CI) = 2.44 (1.25–4.88); $p = 0.005$].

Discussion

We found over half of households had at least one individual colonized with ESCrE. Independent risk factors for ESCrE household colonization included horse/donkey exposure, yogurt consumption, geographic region, and time period. When time period (i.e., pre- vs. post-lockdown) was not included in the model, hospital exposure and pit latrine use were also significant risk factors.

We found the prevalence of households in which at least one person was ESCrE-colonized to be very high (53.8%). In comparison, a recent systematic review showed rates of community ESBL-EB fecal colonization between 2 and 6% in Australia, the Americas and Europe [12]. The high rate of ESCrE household colonization is worrisome as even one colonized individual may serve as a reservoir for transmission to others in the household and the community [17, 18].

The association of ESCrE colonization with exposure to horses has previously been reported [27, 28, 29]. Horses have been shown to be ESCrE carriers, with one study in Turkey finding over 50% of the horses sampled from various farms were colonized with ESCrE. The authors hypothesized this may be due to an overuse of antibiotics in the horse population [29]. In the Netherlands, a study conducted in 2013 assessed risk factors for colonization by ESBL-producing bacteria and found that interacting with a horse increased risk of colonization (OR = 4.69; $p < 0.0001$) [28]. However, specific evidence of zoonotic transmission is lacking [27]. However, these studies do suggest horses can be reservoirs for ESCrE. More specific details on interactions with horses that increase ESCrE colonization risk are needed, as are studies that combine human and animal samples to assess zoonotic transmission characteristics.

This is the first paper to our knowledge to find the association between yogurt consumption and ESCrE colonization. One possible mechanism for this association is ESCrE contamination of the yogurt. In fact, a recent study done in Ethiopia found that among the bacteria isolated from milk and yogurt, ~ 40% were ESBL producers [30]. Specifically, the dairy products were sourced from local farmers, with all individuals not having formal training on how to handle these products hygienically. This suggests possible differences in ESCrE contamination when comparing locally prepared vs. commercially prepared dairy products. In our study, over 95% of households reported obtaining their yogurt from markets. We however did not assess the volume of yogurt consumption in a given individual or household. Future work should seek to assess the possible link between ESCrE and yogurt exposure, distinguishing more precisely the source of yogurt products and the volume of consumption. In addition, it may be useful to sample various sources of dairy including yogurt to determine rates of contamination.

Geographic region, specifically residing in Gaborone, increased risk of ESCrE colonization. This suggests that even within a relatively small geographic region within a country (i.e., study sites are all within 100 km of each other), colonization rates can vary by location. These differences may provide important clues to possible environmental or population-based risk factors for colonization. These geographic differences are also important

to consider when assessing generalizability of a given study's findings.

One of the most unique aspects of the study was the unanticipated COVID-19 lockdown from April 2, 2020, through May 21, 2020. Across Botswana, domestic and international travel was highly limited during this time. Hospital visits by family and friends were also highly restricted. Notably, family often provide considerable assistance in the care of a hospitalized family member. While directed toward control of COVID-19, these restrictions were also associated with ESCrE colonization. There was a significantly lower colonization rate in the post-lockdown period compared to the pre-lockdown period. Both international travel and hospital visitation have been previously associated with ESCrE colonization, so restrictions on these practices may have been drivers of lower colonization rates. It is notable that hospital exposure specifically was a significant risk factor for ESCrE colonization if one did not consider the time period of the study. Once we controlled for whether a household was enrolled during the pre- vs. post-lockdown period, the association between hospital exposure and ESCrE colonization was no longer significant. It is also notable that while we have previously noted a significantly lower overall lower colonization rate of individuals in the post-lockdown period compared to the pre-lockdown period in the same study cohort [24], the median number of household members colonized and the proportion of household members colonized, did not differ pre- vs. post-lockdown. Thus, the country-wide lockdown appears to have had little impact on the proportion of individuals colonized within a household. This is understandable in that the lockdown would have been expected to result in fewer exposures to ESCrE outside the home (e.g., due to travel restrictions), but it would have had no little impact on intra-household exposures.

In our secondary analyses we found that time period (i.e., pre- vs. post-lockdown) and younger median household age were significantly associated with multiple household members being colonized with ESCrE vs. only a single household member. Households in which multiple persons were colonized were also significantly more likely to have individuals < 18 years and < 5 years of age. This suggests that once introduced into a household, ESCrE may be more likely to spread between household members when children are present. It is also possible that children may be the source of ESCrE in the household. Although we did not assess child-care activities in the household, the greater need for toileting care of children, and perhaps less attention to hygiene on the part of children, could help explain ESCrE transmission in the household.

While this study had numerous strengths including the large sample size, comprehensive data collection, and novel focus on LMIC households, there were also several potential limitations. We did not require the full household to be enrolled. Although a median of 71% of eligible household members were enrolled, individual-level data from non-enrolled household members was not available. Further, we did not assess antibiotic use in animals nor did we assess the origin of food sources (e.g., commercial vs. homemade). In addition, we focused only on presence or abscess of ESCrE broadly in the household. Genomic assessment of isolates was not performed in this study nor did we have fecal microbiome samples available for participants. Future work focusing on detailed genomic assessments of both colonizing isolates and the concurrent microbiome would provide valuable insights into the molecular epidemiology of ESCrE colonization and transmission.

In summary, we found numerous independent risk factors for ESCrE household colonization. Further characterization of these risk factors is urgently needed, including more refined epidemiologic determination of exposures (e.g., animal/farm practices), animal sampling, food sampling, longitudinal assessment of ESCrE colonization in households, and genomic characterization of colonizing ESCrE to determine household transmission dynamics. These data hold considerable promise in informing future strategies to curb the emergence of ESCrE in households.

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Author contributions
All authors were involved in the design of the study; KS, MM, and NM enrolled subjects; DO, LG, LC, AP, MRG, MM, GP, and KA conducted laboratory analyses; LC, WB, RG, and EL analyzed and interpreted the patient data; SS, NM, MM, RB, AJB, ES, and EB drafted the manuscript; All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was reviewed and approved by the institutional review boards of the University of Pennsylvania, University of Botswana, and the Botswana Ministry of Health.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Disclaimer
The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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