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Finishing the prescribed course of antibiotics is associated with Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae* carriage – results of a Singapore Community Survey

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ABSTRACT

Objectives: To study the correlation between knowledge, attitude and practices (KAP) of antibiotic consumption with epidemiology and molecular characteristics of ESBL-producing *Enterobacteriaceae* (ESBL-PE) carriage, in order to identify modifiable factors and public health interventions to reduce prevalence of multidrug resistant organism (MDRO) colonisation in the community.

Design: Cross-sectional questionnaire of KAP towards antibiotic use and collection of stool samples or rectal swabs. ESBL-PE isolates obtained underwent whole genome sequencing to identify resistance genes.

Setting: A densely populated community in Singapore

Participants: There were 693 healthy community- dwelling questionnaire respondents. Out of which, 305 provided stool samples or rectal swabs.

Results: The overall knowledge of antibiotic use was poor (mean score 4.6/10, IQR 3.0-6.0). 80 participants (80/305, 26.2%) carried at least one ESBL-PE isolate. The most common ESBL-PE was *E. coli* sequence type 131 carrying CTX-M type beta-lactamases (11/71, 15.5%). Living overseas for more than 1 year (OR 3.3, 95% CI 1.6 to 6.9) but not recent hospitalisation or antibiotic intake was associated with ESBL-PE carriage. Interestingly, higher knowledge scores (OR 2.0, 95%CI 1.03 to 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.2 to 4.9) were independent factors associated with ESBL-PE carriage in the multivariate logistic regression model.

Conclusions: While the role of trans-border transmission of antimicrobial resistance is well known, we may have to examine the current recommendation that all antibiotics courses have to be completed. Clinical trials to determine the optimum duration of treatment for common infections are critically important.

(246 words)

ARTICLE SUMMARY

Strengths and limitations of this study

- Understanding antibiotic consumption behavior of the patients and general public is a research priority in the fight against antimicrobial resistance. Correlation of this behavior with multidrug resistance colonisation at a population level has the potential to influence public health messages and policies but is under-explored.
- Our study found a high prevalence of extended spectrum beta-lactamase producing *Enterobacteriaceae* asymptomatic carriage in a country with strict antibiotic prescription policies, and this is independently associated with not having left over antibiotics.
- To our knowledge, this is the first study that explored antibiotic consumption behavior with the acquisition of MDRO at a community level. This novel approach has the potential to guide clinicians and policy makers in identifying directly actionable interventions for the population.
- The main weakness of our study is that the questionnaire data is self-reported and subjected to recall and interviewer biases. We minimised these errors by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly.
- Given that the minimum effective treatment durations have not been determined for many infections and that a significant proportion of antibiotic prescriptions are inappropriate, the widely accepted message on the necessity to complete antibiotic courses may have to be re-examined.

INTRODUCTION

Multidrug resistant *Enterobacteriaceae* (MDRE) have been identified as "critical priority" resistant organisms by the World Health Organization (WHO) in 2017, and are associated with a high overall all-cause mortality, transmissibility and burden.[1] Resistance is most commonly mediated via the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases.[2] MDRE infections are difficult to treat with few effective antimicrobials on the horizon.[1] Healthy members of the community are increasingly identified as a reservoir of antimicrobial resistance (AMR), especially in the case of ESBL-producing *Enterobacteriaceae* (ESBL-PE).[3] Asymptomatic carriage of ESBL-PE has been associated with more infections, longer hospitalisations, earlier time to death, and higher hospital costs.[4,5] South East Asian (SEA) countries are known to be a hot spot for AMR.[6] However, the region is heterogeneous with varying healthcare standards and antimicrobial stewardship and utilisation policies (ASP).[7] This study aims to correlate the epidemiological and behavioral risk factors of ESBL-PE carriage in Singapore, a high-income country in SEA, as well as delineate the genetic mechanisms associated with these resistant organisms.

METHODOLOGY

Study population

The study was carried out in Clementi Township, a densely populated residential area in the west of Singapore. It comprises 27,142 households with 91,630 residents who are socio-demographically comparable to the general Singapore population in terms of age, gender, ethnicity and housing distribution.[8] From June 2016 to April 2017,

we randomly selected 2,200 households in Clementi for home visits. The study team returned to non-responding households for up to three times on separate days to maximise the response rate. One representative adult above 21 years old in each household was invited to participate in this cross-sectional study; all consenting individuals undertook a questionnaire, while some additionally consented to provide a rectal swab or stool sample. Ethical approval was obtained from National University of Singapore Institutional Review Board (Reference number B-16-245).

Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake and health-seeking behaviour

We conducted a questionnaire study to assess the KAP of participants towards antibiotic use. A 40-item questionnaire was developed after performing a thorough literature review of comparable studies.[9–14] This was then validated by a pilot study involving 75 community-dwelling volunteers to ensure fluency and accuracy in question design and language. A team of thirty-three investigators was trained to administer the survey face-to-face.

The questionnaire comprised four main sections. The first covered socio-demographic data and recent antibiotic intake. The second was an assessment of antibiotic consumption practices, in which two hypothetical scenarios of diarrhoea and upper respiratory tract symptoms were presented, and participants were asked if they would visit the doctor should they experience these symptoms for less than 1 week, if they would expect or insist on an antibiotic prescription from the doctor's visit, and if they would seek a second opinion if antibiotics were not prescribed. The third component assessed participants' attitudes and trust towards primary care healthcare providers,

and was adapted from a validated questionnaire from Hall *et al*.[15] The last component examined participants' knowledge on AMR. The full questionnaire and grading system can be found in Table S1.

Bacterial isolation and antibiotic susceptibility testing

The study team requested fresh stool samples or rectal swabs from all study participants. The samples of those who consented were collected from the participants within 24 hours of production and stored centrally at 0-4°C prior to microbiological processing. All sample processing was carried out in the Singapore General Hospital Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagar™ ESBL* and *CHROMID® CARBA SMART (bioMerieux)* media to detect cephalosporin-resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and used for subsequent species identification and antibiotic susceptibility testing. Species identification was done by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 *(bioMerieux)* system.

Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, cefoxitin, cefepime, amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin, nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin, ertapenem and meropenem were assessed by the disc diffusion method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth generation cephalosporins were identified as potential ESBL producers, while those not susceptible to any carbapenem were identified as potential carbapenemase

producers. Potential carbapenemase producers were tested phenotypically for carbapeneasme production by modified Hodge test and KPC/MBL and OXA-48 Confirm Kit (ROSCO). All potential carbapenease producers were also subjected to the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM carbapemase gene sequences.

Whole genome sequencing of ESBL-producing *Enterobacteriaceae*

DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially ESBL- or carbapenemase- producers, with sequencing libraries for each isolate prepared as per manufacturer's recommendation to be multiplexed sequenced on the Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs, having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads was performed using the SPAdes Genome Assembler.[17] Bacterial species were identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence types (MLSTs) were determined by a customized script utilising BLAST search for identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial resistance owing to the existence of specific gene sequences were performed using SRST2.[20]

Statistical Analysis

Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-PE carriage status and presence of specific resistance genes. Dichotomous variables are expressed in frequencies and percentages, while continuous variables are in means with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric

Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in multivariate analyses to identify statistically significant factors that influence and determine KAP and ESBL-PE carriage. All tests of significance are performed at α=5%. Statistical analysis was carried out using R Version 1.1.383.[21]

Patient and Public Involvement

A group of 75 community dwellers partnered with us for the design and validation of the study questionnaire, production of informational material to support recruitment, and evaluation of the burden of the sample collection from the patient's perspective.

RESULTS

Out of the 2200 households the study team visited, 693 (31.5%) agreed to participate, of whom 305 (44.0%) also provided stool samples or rectal swabs (Figure S1). Participant demographics are presented in Table 1. The median age of participants was 53 (IQR 38-66). A slight majority were women (56.7%). The ethnic distribution of the participants was similar to the wider Singapore population, with 513 (74.0%) Chinese, 78 (11.3%) Malay, and 83 (12.0%) Indian. The majority had received at least secondary school education (534, 77.0%), and stayed in public housing apartments (661, 95.4%). The median number of occupants per household was 3 (IQR 2-4) persons. The vast majority reported having previously taken antibiotics (616, 96.4%) and 102 (14.7%) had recently been hospitalised in the past 1 year.

Table 1. Demographics, medical background and antibiotic use of study participants

Characteristic		N (%) Total N=693
Age (median, IQR*)		53.0 (38.0-66.0)
Females		393 (56.7)
Race	Chinese	513 (74.0)

	Malay	78 (11.3)
	Indian	83 (12.0)
	Other ethnicities	19 (2.7)
Education level	Graduate	88 (12.7)
	Diploma	251 (36.2)
	Secondary	195 (28.1)
	Primary	122 (17.6)
	No Formal Education	37 (5.3)
Housing type	1-, 2 or 3-room public housing	334 (48.2)
	4 or 5- room public housing	327 (47.2)
	Private landed property	32 (4.6)
Number of occupants in the household	Overall (median, IQR)	3 (2-4)
	≤ 3 persons	369 (53.2)
	4-5 persons	257 (37.1)
	≥ 6 persons	67 (9.7)
Comorbidities	Any chronic illnesses	239 (34.5)
	Hypertension	105 (15.2)
	Hyperlipidemia	76 (11.0)
	Diabetes mellitus	67 (9.7)
Recent hospitalisation in the past 1 year		102 (14.7)
Antibiotic consumption	Within past 6 months	175 (25.3)
	More than 6 months ago	441 (63.6)
	Never taken antibiotics	77 (11.1)

*IQR- interquartile range, ^Immunocompromised – Use of chemotherapy, corticosteroids or immunosuppressants in the past 6 months

The survey revealed widespread misinformation about antibiotics, with a mean knowledge score of only 4.6 (IQR 3.0-6.0) out of 10 (Table S2). Although the majority of participants knew that viruses are the most common cause of upper respiratory tract infections, a significant proportion (335/693, 48.3%) believed that antibiotics could be used for viral infections and 385 (385/693, 55.6%) thought that the most common cause of diarrhoea was bacteria. The questionnaire also explored participants' compliance to the traditional view of completing antibiotic courses. The majority (554/693, 79.9%) said they would complete the course of antibiotics prescribed, while 13.7% (95/693) would stop taking antibiotics when they start to feel

better, and 6.3% (44/693) preferred to seek the doctor's opinion before stopping the course. Most participants (564/693, 81.4%) were aware that antibiotics are prescription-only drugs in Singapore, but were unable to correctly answer questions related to AMR, with 82.5% (572/693) not knowing what causes AMR, and 63.2% (438/693) believing AMR was not present in Singapore. The level of education ($p<0.001$) and staying in larger housing ($p=0.037$)—the usual proxy for socio-economic status in Singapore—were independent factors associated with higher total knowledge scores. However, higher knowledge scores were not strongly related to participants' trust in primary care physicians (OR 1.08, 95%CI 0.97-1.20) or the expectation of an antibiotic prescription for common viral infections (OR 0.98, 95%CI 0.96-1.0).

A large majority of the community continued to place trust in their primary care doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted healthcare professionals as their primary source of medical information, over the Internet, media and family and friends. There were no significant associations between demographic factors and attitude scores in contrast to the differences seen in knowledge scores.

In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and vomiting), although about half of the participants (294/693, 42.4% for cough and runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the doctor for common complaints lasting less than 1 week, only 18.5% (average 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not prescribed during the initial visit, very few (average 39/693, 5.6%) reported they

would insist on antibiotic prescription or seek a second opinion. The only independent factor associated with the expectation of an antibiotic prescription was younger age (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3% (23/693) admitted to having previously shared antibiotics with family members and 5.5% (38/693) to having taken leftover antibiotics from a previous illness.

Asymptomatic carriage of ESBL-PE

Three hundred and five participants (305/693, 44.0%) provided rectal swabs or stool samples for microbiology cultures. Eighty participants (80/693, 26.2%, 95%CI: 21.5-31.6%) were found to carry at least one ceftriaxone non-susceptible *Enterobacteriaceae* isolate. One hundred and fifteen isolates were detected on the ESBL screening media, of which 93 were ceftriaxone resistant or intermediate *Enterobacteriaceae*. Six bacterial isolates were detected on the CRE screening media, none of which were confirmed to be carbapenemase-producing *Enterobacteriaceae*. The factors associated with ESBL-PE carriage from multivariate logistic regression analysis were residency overseas for more than 1 year (OR 3.3, 95%CI 1.6-6.9), with the most common location being other parts of Asia, scoring higher than 6 on the knowledge component in the questionnaire (OR 2.0 95%CI 1.03- 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.24-4.9). Interestingly, recent hospitalisation and reported antibiotic intake were not associated with ESBL-PE carriage (Table 2).

Table 2. Risk factors for carriage of ceftriaxone- resistant *Enterobactriaceae*

Factors		Total N=305	Carriers N=80	Non-carriers N=225	p-values
Age (median, IQR*)		54.0 (41.0-65.0)	56.0 (38.8-66.0)	54.0 (41.0-65.0)	0.79
Females (%)		169 (55.4)	46 (57.5)	123 (54.7)	0.76
Ethnicity (%)	Chinese	237 (77.7)	67 (83.8)	170 (75.6)	0.24
	Malay	28 (9.2)	3 (3.8)	25 (11.1)	
	Indian	30 (9.8)	7 (8.8)	23 (10.2)	
	Others	10 (3.3)	3 (3.8)	7 (3.1)	
Education (%)	No formal education	11 (3.6)	4 (5.0)	7 (3.1)	0.45
	Primary	57 (18.7)	12 (15.0)	45 (20.0)	
	Secondary	93 (30.5)	21 (26.2)	72 (32.0)	
	Tertiary	110 (36.1)	31 (38.8)	79 (35.1)	
	Graduate	34 (11.1)	12 (15.0)	22 (9.8)	
Housing (%)	HDB 1- and 2-room	23 (7.5)	5 (6.2)	18 (8.0)	0.75
	HDB 3-room	115 (37.7)	32 (40.0)	83 (36.9)	
	HDB 4-room	98 (32.1)	24 (30.0)	74 (32.9)	
	HDB 5-room and Executive Apartment	47 (15.4)	11 (13.8)	36 (16.0)	
	Landed Property	22 (7.2)	8 (10.0)	14 (6.2)	
Pets (%)		33 (10.8)	7 (8.8)	26 (11.6)	0.75
Number of occupants in the household (mean, sd)		3.6 (1.6)	3.6 (1.6)	3.6 (1.6)	0.71
Stayed overseas for >1 year (%)		57 (18.7)	26 (32.5)	31 (13.8)	<0.001
Stayed in South, East or Southeast Asia for >1 year (%)		40 (13.1)	18 (22.5)	22 (9.8)	0.007
Travelled in the past >1 year (%)		178 (58.4)	47 (58.8)	131 (58.2)	1.0
Travelled in South, East or Southeast Asia in the past 1 year (%)		163 (53.4)	43 (53.8)	120 (53.3)	1.0
Any chronic illnesses (%)		127 (41.6)	33 (41.2)	94 (41.8)	1.0
Hospitalisation in the past 1 year (%)		43 (14.1)	14 (17.5)	29 (12.9)	0.41
Previous antibiotics intake (%)		282 (92.5)	76 (95.0)	206 (91.6)	0.45
Antibiotics in the last 6 months		85 (27.9)	23 (28.8)	62 (27.6)	0.61

(%)				

*IQR- interquartile range

Out of the 93 ceftriaxone-resistant isolates, 17 were cefoxitin resistant, suggestive of AmpC β-Lactamase production. Only one *Enterobacter cloacae* complex isolate was resistant to ertapenem and was of intermediate susceptibility to meropenem (Table 3). This *Enterobacter cloacae* complex isolate was not a carbapenemase-producer based on phenotypic and genotypic tests. Eighty-three (83/93, 89.2%) of these ESBL-PE isolates were *E. coli*. The majority of ESBL-PE remained susceptible to aminoglycosides including gentamicin (80/93, 86.0%) and amikacin (91/93, 97.8%) as well as nitrofurantoin (76/93, 81.7%), while ciprofloxacin (53/93, 57.0%) and Sulfamethoxazole-trimethoprim (32/93, 34.4%) resistance were more common.

Table 3. Antibiotic susceptibility of the ceftriaxone-resistant isolates

	E coli (N=83) N (%)	*Klebsiella* (N=6) N (%)	Others^ (N=4) N (%)	Total (N=93) N (%)
Piperacillin-tazobactam	73 (88.0)	4 (66.7)	1 (25.0)	78 (83.9)
Cefepime	35 (42.4)	3 (50)	2 (50.0)	40 (43.0)
Aztreonam	39 (47.0)	2 (33.3)	1 (25.0)	42 (45.2)
Amikacin	82 (98.8)	5 (83.3)	4 (100)	91 (97.8)
Gentamicin	75 (90.4)	3 (50)	2 (50.0)	80 (86.0)
Nitrofuratoin	73 (88.0)	2 (33.3)	1 (25.0)	76 (81.7)
Sulfamethoxazole-trimethoprim	32 (38.6)	0 (0)	0 (0)	32 (34.4)
Ciprofloxacin	48 (57.8)	4 (66.7)	1 (25.0)	53 (57.0)
Fosfomycin	63 (75.9)	1 (16.7)	0 (0)	64 (68.8)
Ertapenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)
Meropenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)

^ Others include *Enterobacter* spp (2), *Proteus mirabillis* (1), *Raoultella ornithinolyitca* (1)

Molecular classification of ESBL-PE

Eighty (80/93, 85%) ESBL-PE isolates from unique participants underwent whole genome sequencing. When two or more isolates grew from a single subject's sample, *E. coli*, the commonest species observed, was selected to facilitate comparisons. Genotypic species determination from the sequence reads correlated completely with the results by MALDI-TOF MS or the Vitek-2 system. Seventy-one (71/80, 88.8%) isolates were *E. coli*, of which the most common molecular type was sequence type (ST) 131 (11/71, 15.5%) (Table 4). The most frequently observed ESBL gene was CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27 (16/71, 22.5%). More *E coli* ST131 were resistant to fluoroquinolones than non-ST131 isolates (p=0.041). The only significant factor from the questionnaire associated with ESBL-producing *E. coli* ST131 carriage was having more children in the household, but the difference was marginal (mean 0.3±0.7 versus 0.8 ± 1.1, p=0.034).

Table 4. Molecular classification of ceftriaxone-resistant E coli isolates

		E coli N=71 (%)		p-value
		ST131 N=11 (%)	Non ST131 N=60 (%)	
Number of resistant genes (mean ± sd)		1.2 ± 0.4	1.9 ± 0.8	0.0012
ESBL genes				
CTXM	15	4 (36.4)	17 (28.3)	0.72
	27	7 (63.6)	9 (15.0)	
	14	0 (0.0)	10 (16.7)	
	55	0 (0.0)	9 (15.0)	
	8	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	9 (15.0)	

	None	0 (0.0)	3 (5.0)	
SHV	12	0 (0.0)	3 (5.0)	1.0
	None	11 (100.0)	57 (95.0)	
TEM	206	1 (9.1)	11 (18.3)	0.11
	198	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	15 (25.0)	
	None	10 (90.9)	31 (51.7)	
OXA		1 (9.1)	3 (5.0)	1.0
Quinolone resistance		8 (72.7)	21 (35.0)	0.041

* Non-ST131 sequence types are: 38 (N=8), 1193 (N=5), 10 (N=4), 48 (N=3), other (N=35), none (N=5)

DISCUSSION

We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy community dwellers in Singapore, twice the rate found in an earlier study in 2014 of patients at an emergency department.[22] Similar rises have been observed globally.[3] Although these figures are lower than the reported prevalence of over 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly regulated antibiotic prescription system similar to Europe and the US where only registered medical practitioners are allowed to prescribe antibiotics, and they must be purchased from licensed dispensers. We did not find any association between fecal carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a city-state and overseas travel is very common, making it hard to detect such a relationship when frequent trips to neighbouring countries are made. However, past residency overseas was strongly associated with colonisation, especially those who lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). The possibility of substantial acquisition of MDRO colonisation and infection through overseas

exposure[24,25] once again highlights the urgent need for a regional, collaborative approach to tackling the problem of AMR.

Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL mechanism, echoing the global dissemination of this hyperendemic clone, especially in the community.[26] Similar to reports from communities was 11.1% (32/287) in China[27] and 4.1% (8/193) in Thailand[28] have been published. The reason for the rapid worldwide expansion and long-term persistence of *E. coli* ST131 is thought to be due to compensatory mutations within the core genome counterbalancing the fitness cost associated with IncF plasmids, thus sustaining its spread even in the absence of direct antibiotic selection pressure.[29] These *E. coli* ST131 are not just prevalent colonisers but have also associated with invasive bloodstream infections in hospitalized patients in Australia, New Zealand and Singapore.[30] It will be important to better understand the evolutionary ecology and transmission dynamics of this emerging clone.

This study also revealed widespread misconceptions about the utility of antibiotics for viral infections, consistent with the findings of a global survey conducted by the WHO in 2015.[31] We also found that, the public continues to place trust in their primary care doctors and their recommendations. This dependence on physicians is in contrast to doctors' perceptions of patient expectations for antibiotic prescriptions.[32] This discordance has been previously described and is thought to be due to the lack of empowerment of the patient and the erroneous attribution of patient

satisfaction to antibiotic prescription rather than a focus on better patient-doctor communication.[33,34]

Engaging and educating both the prescribers and the public may reduce inappropriate antibiotic use,[35,36] and has been identified as a key strategy by the WHO and the UK to tackle AMR.[37,38] One of the most striking findings of this study is that having a higher knowledge score and not having left over antibiotics were independent risk factors for carriage of ESBL-PE. The current WHO recommendation remains that full courses of antibiotics should be completed to prevent the onset of resistance.[31] Similar messages are advocated in national campaigns launched in Australia,[39] Canada,[40] the United States[41] and Europe.[42] However, increasing evidence is emerging supporting shorter duration of antibiotics for common infections.[43] The impact of prolonged antibiotic use on the host flora is often underestimated.[43] Given that the minimum effective treatment durations have not been determined for many infections and that a significant proportion of antibiotic prescriptions are inappropriate, the whole question about "completing the course" of antibiotics may have to be re-examined.

To our knowledge, this is the first study that explored antibiotic consumption behavior with the acquisition of MDRO at a community level. This novel approach has the potential to guide clinicians and policy makers in identifying directly actionable interventions for the population. The main weakness of our study is that the questionnaire data is self-reported and subjected to recall and interviewer biases. We minimised these errors by designing specific questions that are carefully

constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly.

CONCLUSION

There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore, especially with *E. coli* ST131 carrying CTX-M. Innovative approaches to control AMR that take into account transboundary transmission of resistance and clinical trials to determine the appropriate duration of antimicrobial therapy will be critical to control the emergence of these resistant clones which have contributed significantly to the current global antibiotic resistance crisis.

CONTRIBUTOR AND GUARANTOR INFORMATION

YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK performed microbiological testing. RTHO planned and conducted genomic sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW performed data analysis. All participated in the writing of the script, and affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained. YM and IS accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and no others meeting the criteria have been omitted.

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COMPETING INTERESTS DECLARATION

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

DATA SHARING

The authors commit to making the relevant anonymised patient level data available on reasonable request.

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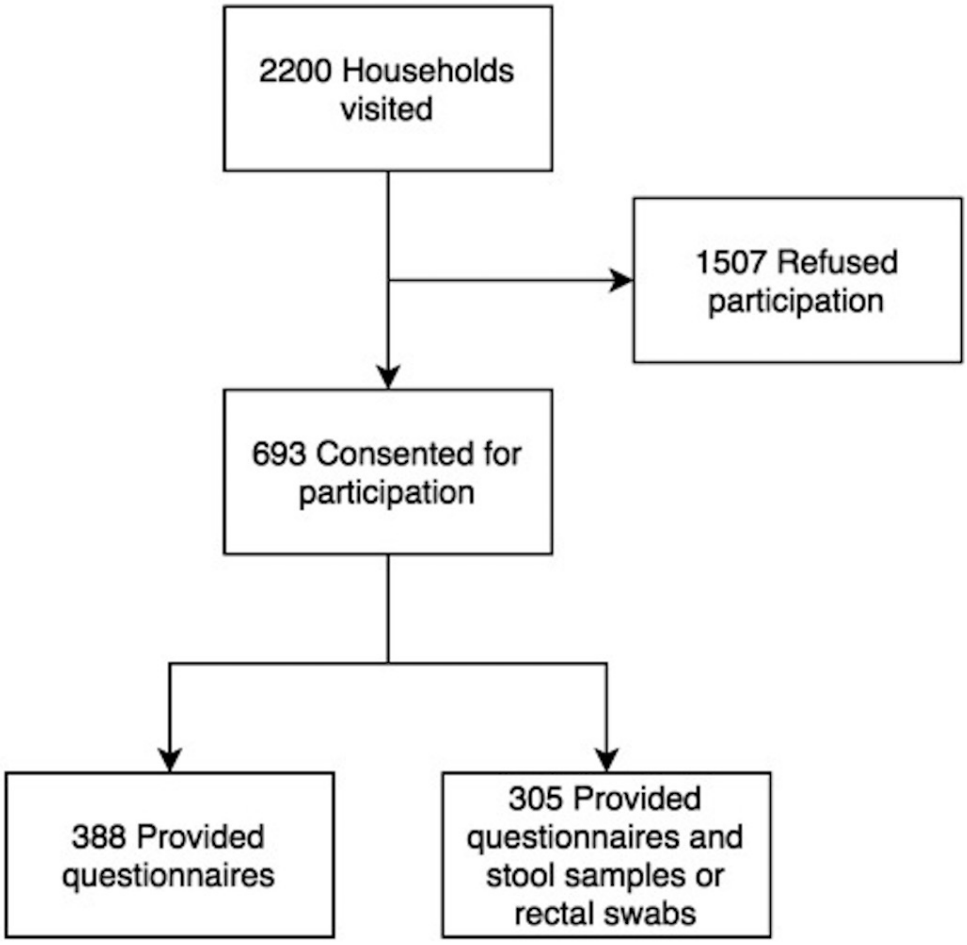

Figure S1. Participant recruitment flowchart

90x87mm (300 x 300 DPI)

Table S1. Study questionnaire

Section 1: Background Data

1. Demographic Data
1.1 Age
1.2 Gender - Male or Female
2. Race - Chinese or Malay or Indian or Others
3. Educational Background
2.1 Highest Education Level Attained- Primary Education or Secondary Education or Tertiary Education or Graduate Education or No formal education
4. Have you ever studied a healthcare-related course? (Medicine, Traditional Chinese Medicine, Therapy, Nursing) - Yes or No
3 Occupation and Financial Status
5. Ocupation:
4 Accommodation
4.1 Housing type- Public housing (1-Room or 2-Room or 3-Room or 4-Room or 5-Room or Executive Apartment) or Landed property
4.2 How many occupants are there living in your house? (including you) Number of Occupants:
4.3.1 How many people in the household are in the following age group? Less than 12 years old:
4.3.2 How many people in the household are in the following age group? More than 65 years old:
6. Do you currently have any dogs or cats at home? - Yes or No
5 Travel history

5.3 Have you travelled to the following places within the past 6 months? – Yes or No

5.3.1 If yes, which of the following places have you been to? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

5.4 Have you lived anywhere else for more than 1 year? – Yes or No

6 If yes, did you live in the following areas? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

7 Medical History

6.1 Do you have any of the following? (You can choose more than one of the following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids, Immunosuppressants etc) and/ or Other medical conditions or None of the above

6.2 When was your last hospitalisation? - Never been hospitalised before or Hospitalised before

6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

6.2.2 How long was your stay? Duration:

6.3 Have you used antibiotics before? - Have never used antibiotics before or Used antibiotics before

6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6

months or More than 6 months ago

Section 2: Assessment of Antibiotic Practices

1. Assessing Health-Seeking and Antibiotic-Seeking Behaviours

Scenario 1: Cough and Runny Nose

1.1.1 Would you go to the doctor for a cough and runny nose that lasted less than 1 week? – Yes or No or I am not sure

1.1.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.1.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

Scenario 2: Diarrhoea and Vomiting

1.2.1 Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than a week? – Yes or No or I am not sure

1.2.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.2.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

2. Assessing Practices of Disposal and Storage of Antibiotics

2.1 What do you usually do with leftover antibiotics? - Usually do not have leftovers

or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the rubbish bin or Others:

3. Assessing Alternative Antibiotic Practices

3.1 Have you ever shared antibiotics with someone else? – Yes or No

3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes or No

Section 3: Attitude Assessment

1. Attitudes Towards Healthcare Provider Prescription

1.1 Sometimes my doctor prioritises what is beneficial for him over my medical needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.3 My doctor is always honest when telling me about all the available treatments for my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

2. Attitudes Towards Potential Educational Interventions

2.1 Which of the following sources of medical information do you trust most? - Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists) or Family and Friends or Online Medical Sources or Television Programmes and Advertisements or Radio Programmes and Advertisements

Section 4: Knowledge Assessment

1. Knowledge on Function of Antibiotics

1.1 Antibiotics are medicines that can treat viral infections. – True or False or I am not sure

1.2 Antibiotics are medicines that can treat bacterial infections. – True or False or I am not sure

1.3 Antibiotics are medicines that can treat fungal infections. – True or False or I am not sure

2. Knowledge on Agents of Infection

2.1 Which of the following most commonly causes running nose and cough? – Virues or Bacteria or I am not sure

2.2 Which of the following most commonly causes diarrhoea? – Virues or Bacteria or I am not sure

3. Knowledge on Proper Use of Antibiotics

3.1 Antibiotics can be obtained at the pharmacist without any prescription. - True or False or I am not sure

3.2 Antibiotics can be stopped when: - You start to feel better or You finish the entire course or You head back to the doctor and he tells you that you can stop

4. Knowledge on Concept of Antibiotic Resistance

4.1 Do you understand what is antibiotic resistance? – Yes or No or I am unsure

4.1.1 If yes, describe what causes antibiotic resistance?

4.2 Which of the following is a consequence of antibiotic resistance? (choose only ONE option) - Antibiotics become more effective at treating infections or Antibiotics become less effective at treating infections or Your body immunity

becomes weaker or Your body immunity becomes stronger

4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am unsure

Table S2. Assessment of knowledge

Questions			N (%) Total N= 693
2.1.1	Antibiotics are medicines that can treat viral infections.	False	149 (21.5%)
		True	335 (48.3%)
		Unsure	209 (30.2%)
2.1.2	Antibiotics are medicines that can treat bacterial infections.	True	419 (60.5%)
		False	50 (7.2%)
		Unsure	224 (32.3%)
2.1.3	Antibiotics are medicines that can treat fungal infections.	False	157 (22.7%)
		True	194 (28.0%)
		Unsure	342 (49.4%)
2.1.4	Which of the following most commonly causes running nose and cough.	Viruses	352 (50.8%)
		Bacteria	130 (18.8%)
		Unsure	211 (30.4%)
2.1.5	Which of the following most commonly causes diarrhoea?	Viruses	98 (14.1%)
		Bacteria	385 (55.6%)
		Unsure	210 (30.3%)
2.1.6	Antibiotics can be stopped when…	You finish the entire course	554 (79.9%)
		When you feel better	95 (13.7%)
		Consult the doctor	44 (6.3%)
2.1.7	Antibiotics can be obtained at the pharmacist without any prescription.	False	564 (81.4%)
		True	29 (4.2%)
		Unsure	100 (14.4%)

2.1.8	What causes antimicrobial resistance? (Open ended)	Inappropriate use of antibiotics	121 (17.5%)
		Wrong or unsure	572 (82.5%)
2.1.9	Which of the following is a consequence of antibiotic resistance?	Antibiotics becoming more effective at treating infections	280 (40.4%)
		Antibiotics becoming less effective at treating infections	111 (16.0%)
		Your body immunity becomes weaker	235 (33.9%)
		Your body immunity becomes stronger	67 (9.7%)
2.1.10	Antibiotic resistance is not present in Singapore yet.	False	255 (36.8%)
		True	77 (11.1%)
		Unsure	361 (52.1%)

Table S3. Assessment of attitude toward primary care

Questions			N (%) N= 693
2.2.1	Sometimes my doctor prioritises what is beneficial for him over my medical needs	Strongly agree	14 (2.0)
		Agree	109 (15.7)
		Neutral	145 (20.9)
		Disagree	335 (48.3)
		Strongly disagree	90 (13.0)
2.2.2	My doctor's medical skills are not as good as they should be	Strongly agree	10 (1.4)
		Agree	83 (12.0)
		Neutral	150 (21.6)
		Disagree	373 (53.8)
		Strongly disagree	77 (11.1)
2.2.3	My doctor is always honest when telling me about all the available treatments for my condition	Strongly agree	100 (14.4)
		Agree	427 (61.6)
		Neutral	115 (16.6)
		Disagree	45 (6.5)
		Strongly disagree	6 (0.9)
2.2.4	I have no worries about putting my life in my doctor's hands	Strongly agree	110 (15.9)
		Agree	363 (52.4)
		Neutral	135 (19.5)
		Disagree	74 (10.7)
		Strongly disagree	11 (1.6)
2.2.5	Which of the following sources of medical information do you trust most?	Healthcare professional's advice	627 (90.6)
		Family and friends	36 (5.2)
		Online medical sources	24 (3.5)

		Television programmes and advertisements	4 (0.6)
		Radio programmes and advertisements	1 (0.1)

Table S4. Assessment of practices

Questions			N (%) Total N= 693
2.3.1.1	Would you go to the doctor for a cough and runny nose that lasted less than 1 week	Yes	294 (42.4)
		No	377 (54.4)
		Unsure	22 (3.2)
2.3.1.2	Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than 1 week?	Yes	414 (59.7)
		No	262 (37.8)
		Unsure	17 (2.5)
2.3.2.1	Would you expect the doctor to prescribe antibiotics for cough and runny nose that lasted less than 1 week to help with the recovery?	Yes	136 (19.6)
		No	508 (73.3)
		Unsure	49 (7.1)
2.3.2.2	Would you expect the doctor to prescribe antibiotics for diarrhoea, vomiting and stomach pain that lasted less than 1 week to help with the recovery?	Yes	120 (17.3)
		No	501 (72.3)
		Unsure	72 (10.4)
2.3.3.1	If the doctor you were seeing does not prescribe you antibiotics for cough and runny nose that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	37 (5.3)
		No	619 (89.3)
		Unsure	37 (5.3)
2.3.3.2	If the doctor you were seeing does not prescribe you antibiotics for diarrhea vomiting and stomach pain that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	40 (5.8)
		No	615 (88.7)
		Unsure	38 (5.5)
2.3.4.1	What do you usually do with left over antibiotics?	No left overs	476 (68.7)
		Disposal in rubbish bin	130 (18.8)
		Keep for future use	60 (8.7)

		Unsure	19 (2.7)
		Pour down sink or toilet bowl	8 (1.2)
2.3.4.2	Have you ever shared antibiotics with anyone else?	Yes	23 (3.3)
		No	670 (94.5)
2.3.4.3	Have you ever taken leftover antibiotics from a previous course of illness?	Yes	38 (5.5)
		No	655 (9.5)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-8
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	4-5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Supplementary material
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	9-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-15
		(*b*) Report category boundaries when continuous variables were categorized	9-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19-20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Relating knowledge, attitude and practice of antibiotic use to extended spectrum Beta-Lactamase producing Enterobacteriaceae carriage – results of a Singapore Community Survey

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Relating knowledge, attitude and practice of antibiotic use to extended spectrum Beta-Lactamase producing *Enterobacteriaceae* carriage – results of a Singapore Community Survey

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ABSTRACT

Objectives: To study the correlation between knowledge, attitude and practices (KAP) of antibiotic consumption with epidemiology and molecular characteristics of ESBL-producing *Enterobacteriaceae* (ESBL-PE) carriage, in order to identify modifiable factors and public health interventions to reduce prevalence of multidrug resistant organism (MDRO) colonisation in the community.

Design: Cross-sectional questionnaire of KAP towards antibiotic use and collection of stool samples or rectal swabs. ESBL-PE isolates obtained underwent whole genome sequencing to identify resistance genes.

Setting: A densely populated community in Singapore

Participants: There were 693 healthy community- dwelling questionnaire respondents. Out of which, 305 provided stool samples or rectal swabs.

Results: The overall knowledge of antibiotic use was poor (mean score 4.6/10, IQR 3.0-6.0). 80 participants (80/305, 26.2%) carried at least one ESBL-PE isolate. The most common ESBL-PE was *E. coli* sequence type 131 carrying CTX-M type beta-lactamases (11/71, 15.5%). Living overseas for more than 1 year (OR 3.3, 95% CI 1.6 to 6.9) but not short-term travel, recent hospitalisation or antibiotic intake was associated with ESBL-PE carriage. Interestingly, higher knowledge scores (OR 2.0, 95%CI 1.03 to 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.2 to 4.9) were independent factors associated with ESBL-PE carriage in the multivariate logistic regression model.

Conclusions: While the role of trans-border transmission of antimicrobial resistance is well known, we may have to examine the current recommendation that all antibiotics courses have to be completed. Clinical trials to determine the optimum duration of treatment for common infections are critically important.

(246 words)

ARTICLE SUMMARY

Strengths and limitations of this study

- Understanding antibiotic consumption behavior of the patients and general public is a research priority in the fight against antimicrobial resistance. Correlation of this behavior with multidrug resistance colonisation at a population level has the potential to influence public health messages and policies but is under-explored.
- Our study found a high prevalence of extended spectrum beta-lactamase producing *Enterobacteriaceae* asymptomatic carriage in a country with strict antibiotic prescription policies, and this is independently associated with not having left over antibiotics.
- To our knowledge, this is the first study that explored antibiotic consumption behavior with the acquisition of MDRO at a community level. This novel approach has the potential to guide clinicians and policy makers in identifying directly actionable interventions for the population.
- The main weakness of our study is that the questionnaire data is self-reported and subjected to recall and interviewer biases. We minimised these errors by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly.
- Given that the minimum effective treatment durations have not been determined for many infections and that a significant proportion of antibiotic prescriptions are inappropriate, the widely accepted message on the necessity to complete antibiotic courses to reduce antibiotic resistance may have to be re-examined.

INTRODUCTION

Multidrug resistant *Enterobacteriaceae* (MDRE) have been identified as "critical priority" resistant organisms by the World Health Organization (WHO) in 2017, and are associated with a high overall all-cause mortality, transmissibility and burden.[1] Resistance in *Enterobacteriaceae* is most commonly mediated via the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases.[2] MDRE infections are difficult to treat with few effective antimicrobials on the horizon.[1] Healthy members of the community are increasingly identified as a reservoir of antimicrobial resistance (AMR), especially in the case of ESBL-producing *Enterobacteriaceae* (ESBL-PE).[3] Asymptomatic carriage of ESBL-PE has been associated with more infections, longer hospitalisations, earlier time to death, and higher hospital costs.[4,5]

South East Asian (SEA) countries are known to be a hot spot for AMR.[6] However, the region is heterogeneous with varying healthcare standards and antimicrobial stewardship and utilisation policies.[7] To aid in designing effective public health policies and engage the community in the campaign against AMR, it is crucial to understand the local knowledge, attitude and practices of antibiotic use. This study aims to correlate the epidemiological and behavioral risk factors of ESBL-PE carriage in Singapore, a high-income country in SEA, as well as delineate the genetic mechanisms associated with these resistant organisms.

METHODOLOGY

Study population

The study was carried out in Clementi Township, a densely populated residential area in the west of Singapore. It comprises 27,142 households with 91,630 residents who are socio-demographically comparable to the general Singapore population in terms of age, gender, ethnicity and housing distribution.[8] The study team returned to non-responding households for up to three times on separate days to maximise the response rate. The first adult above 21 years old in each household who responded to the study team was invited to participate in this cross-sectional study; all consenting individuals undertook a questionnaire, while some additionally consented to provide a rectal swab or stool sample. To calculate the number of samples required to estimate the prevalence of ESBL-PE in the community, we used one-sample Z-test with an estimated prevalence of 50%, a confidence interval of 95% and maximum tolerable error of 10%. This yielded about 100 stool samples. Ethical approval was obtained from National University of Singapore Institutional Review Board (Reference number B-16-245).

Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake and health-seeking behaviour

We conducted a questionnaire study to assess the KAP of participants towards antibiotic use. A 40-item questionnaire was developed after performing a thorough literature review of comparable studies.[9–14] This was then validated by a pilot study involving 75 community-dwelling volunteers to ensure fluency and accuracy in question design and language. A team of thirty-three investigators was trained to administer the survey face-to-face, in languages that the participants are fluent in with standardised explanations, to ensure consistency.

The questionnaire comprised four main sections. The first covered socio-demographic data and recent antibiotic intake. The second was an assessment of antibiotic consumption practices, in which two hypothetical scenarios of diarrhoea and upper respiratory tract symptoms were presented, and participants were asked if they would visit the doctor should they experience these symptoms for less than 1 week, if they would expect or insist on an antibiotic prescription from the doctor's visit, and if they would seek a second opinion if antibiotics were not prescribed. The third component assessed participants' attitudes and trust towards primary care healthcare providers, and was adapted from a validated questionnaire from Hall *et al*.[15] The last component examined participants' knowledge on AMR. The full questionnaire and grading system can be found in Table S1.

Bacterial isolation and antibiotic susceptibility testing

The study team requested fresh stool samples or rectal swabs from all study participants. The samples of those who consented were collected from the participants within 24 hours of production and stored centrally at 0-4°C prior to microbiological processing. All sample processing was carried out in the Singapore General Hospital Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagar™ ESBL* and *CHROMID® CARBA SMART (bioMerieux)* media to detect cephalosporin-resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and used for subsequent species identification and antibiotic susceptibility testing. Species identification was done by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 *(bioMerieux)* system.

Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, cefoxitin, cefepime, amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin, nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin, ertapenem and meropenem were assessed by the disc diffusion method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth generation cephalosporins were identified as potential ESBL producers, while those not susceptible to any carbapenem were identified as potential carbapenemase producers. Potential carbapenemase producers were tested phenotypically for carbapeneasme production by modified Hodge test and KPC/MBL and OXA-48 Confirm Kit (ROSCO). All potential carbapenease producers were also subjected to the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM carbapemase gene sequences.

Whole genome sequencing of ESBL-producing *Enterobacteriaceae*

DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially ESBL- or carbapenemase- producers, with sequencing libraries for each isolate prepared as per manufacturer's recommendation to be multiplexed sequenced on the Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs, having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads was performed using the SPAdes Genome Assembler.[17] Bacterial species were identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence types (MLSTs) were determined by a customized script utilising BLAST search for identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial

resistance owing to the existence of specific gene sequences were performed using SRST2.[20]

Statistical Analysis

Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-PE carriage status and presence of specific resistance genes. Dichotomous variables are expressed in frequencies and percentages, while continuous variables are in means with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in multivariate analyses to identify statistically significant factors that influence and determine KAP and ESBL-PE carriage. Covariates that were found to be statistically significant in the univariate analyses were included in the multivariate models. All tests of significance are performed at α=5%. Statistical analysis was carried out using R Version 1.1.383.[21]

Patient and Public Involvement

A group of 75 community dwellers partnered with us for the design and validation of the study questionnaire to ensure clarity and accuracy, production of informational material to support recruitment, and evaluation of the burden of the sample collection from the patient's perspective. Because there was no clear preference for the sample collection methodology, the study team decided to offer both options of rectal swab and stool collection to the study participants.

RESULTS

From June 2016 to April 2017, we randomly selected 2,200 households in Clementi for home visits. Out of these 2200 households, 693 (31.5%) agreed to participate, of whom 305 (44.0%) also provided stool samples or rectal swabs (Figure S1). Participant demographics are presented in Table 1. The median age of participants was 53 (IQR 38-66). A slight majority were women (393/693, 56.7%). The ethnic distribution of the participants was similar to the wider Singapore population, with 513 (74.0%) Chinese, 78 (11.3%) Malay, and 83 (12.0%) Indian. The majority had received at least secondary school education (534/693, 77.0%), and stayed in public housing apartments (661/693, 95.4%). The median number of occupants per household was 3 (IQR 2-4) persons. A quarter (25.3%, 175/693) reported having taken antibiotics in the past 6 months, and 102 (14.7%) had recently been hospitalised in the past 1 year.

Table 1. Demographics, medical background and antibiotic use of study participants

Characteristic		N (%) Total N=693
Age (median, IQR*)		53.0 (38.0-66.0)
Females		393 (56.7)
Race	Chinese	513 (74.0)
	Malay	78 (11.3)
	Indian	83 (12.0)
	Other ethnicities	19 (2.7)
Education level	Graduate	88 (12.7)
	Diploma	251 (36.2)
	Secondary	195 (28.1)
	Primary	122 (17.6)
	No Formal Education	37 (5.3)
Housing type	1-, 2 or 3-room public housing	334 (48.2)
	4 or 5- room public housing	327 (47.2)
	Private landed property	32 (4.6)
Number of occupants in the household	Overall (median, IQR)	3 (2-4)
	≤ 3 persons	369 (53.2)
	4-5 persons	257 (37.1)
	≥ 6 persons	67 (9.7)

Comorbidities	Any chronic illnesses	239 (34.5)
	Hypertension	105 (15.2)
	Hyperlipidemia	76 (11.0)
	Diabetes mellitus	67 (9.7)
Recent hospitalisation in the past 1 year		102 (14.7)
Antibiotic consumption	Within past 6 months	175 (25.3)
	More than 6 months ago	441 (63.6)
	Never taken antibiotics	77 (11.1)

*IQR- interquartile range, ^Immunocompromised – Use of chemotherapy, corticosteroids or immunosuppressants in the past 6 months

The survey revealed widespread misinformation about antibiotics, with a mean knowledge score of only 4.6 (IQR 3.0-6.0) out of 10 (Table S2). Although the majority of participants knew that viruses are the most common cause of upper respiratory tract infections, a significant proportion (335/693, 48.3%) believed that antibiotics could be used for viral infections and 385 (385/693, 55.6%) thought that the most common cause of diarrhoea was bacteria. The questionnaire also explored participants' compliance to the widely accepted view of completing antibiotic courses. The majority (554/693, 79.9%) said they would complete the course of antibiotics prescribed, while 13.7% (95/693) would stop taking antibiotics when they start to feel better, and 6.3% (44/693) preferred to seek the doctor's opinion before stopping the course. Most participants (564/693, 81.4%) were aware that antibiotics are prescription-only drugs in Singapore, but were unable to correctly answer questions related to AMR, with 82.5% (572/693) not knowing what causes AMR, and 63.2% (438/693) believing AMR was not present in Singapore. Level of education ($p<0.001$) and staying in larger housing ($p=0.037$)—the usual proxies for socio-economic status in Singapore—were independent factors associated with higher total knowledge scores. However, higher knowledge scores were not strongly related to participants' trust in primary care physicians (OR 1.08, 95%CI 0.97-1.20) or the

expectation of an antibiotic prescription for common viral infections (OR 0.98, 95%CI 0.96-1.0).

A large majority of the community continued to place trust in their primary care doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted healthcare professionals as their primary source of medical information, over the Internet, media and family and friends. There were no significant associations between demographic factors and attitude scores in contrast to the differences seen in knowledge scores.

In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and vomiting), although about half of the participants (294/693, 42.4% for cough and runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the doctor for common complaints lasting less than 1 week, only 18.5% (average 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not prescribed during the initial visit, very few (average 39/693, 5.6%) reported they would insist on antibiotic prescription or seek a second opinion. The only independent factor associated with the expectation of an antibiotic prescription was younger age (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3% (23/693) admitted to having previously shared antibiotics with family members and 5.5% (38/693) to having taken leftover antibiotics from a previous illness.

Asymptomatic carriage of ESBL-PE

Three hundred and five participants (305/693, 44.0%) provided rectal swabs or stool samples for microbiology cultures. The participants who provided stool samples were not significantly different from those who did not, in terms of age, gender and education level. Eighty participants (80/305, 26.2%, 95%CI: 21.5-31.6%) were found to carry at least one ceftriaxone non-susceptible *Enterobacteriaceae* isolate. One hundred and fifteen isolates were detected on the ESBL screening media, of which 93 were ceftriaxone resistant or intermediate *Enterobacteriaceae*. Six bacterial isolates were detected on the CRE screening media, none of which were confirmed to be carbapenemase-producing *Enterobacteriaceae*. The factors associated with ESBL-PE carriage from multivariate logistic regression analysis were residency overseas for more than 1 year (OR 3.3, 95%CI 1.6-6.9), with the most common location being other parts of Asia, scoring higher than 6 on the knowledge component in the questionnaire (OR 2.0 95%CI 1.03- 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.24-4.9). Interestingly, recent hospitalisation and reported antibiotic intake were not associated with ESBL-PE carriage (Table 2).

Table 2. Univariate analysis of demographic characteristics associated with carriage of ceftriaxone- resistant *Enterobactriaceae*

Factors		Total N=305	Carriers N=80	Non-carriers N=225	p-values
Age (median, IQR*)		54.0 (41.0-65.0)	56.0 (38.8-66.0)	54.0 (41.0-65.0)	0.79
Females (%)		169 (55.4)	46 (57.5)	123 (54.7)	0.76
Ethnicity (%)	Chinese	237 (77.7)	67 (83.8)	170 (75.6)	0.24
	Malay	28 (9.2)	3 (3.8)	25 (11.1)	
	Indian	30 (9.8)	7 (8.8)	23 (10.2)	
	Others	10 (3.3)	3 (3.8)	7 (3.1)	
Education (%)	No formal	11 (3.6)	4 (5.0)	7 (3.1)	0.45

	education				
	Primary	57 (18.7)	12 (15.0)	45 (20.0)	
	Secondary	93 (30.5)	21 (26.2)	72 (32.0)	
	Tertiary	110 (36.1)	31 (38.8)	79 (35.1)	
	Graduate	34 (11.1)	12 (15.0)	22 (9.8)	
Housing (%)	HDB 1- and 2-room	23 (7.5)	5 (6.2)	18 (8.0)	0.75
	HDB 3-room	115 (37.7)	32 (40.0)	83 (36.9)	
	HDB 4-room	98 (32.1)	24 (30.0)	74 (32.9)	
	HDB 5-room and Executive Apartment	47 (15.4)	11 (13.8)	36 (16.0)	
	Landed Property	22 (7.2)	8 (10.0)	14 (6.2)	
Pets (%)		33 (10.8)	7 (8.8)	26 (11.6)	0.75
Number of occupants in the household (mean, sd)		3.6 (1.6)	3.6 (1.6)	3.6 (1.6)	0.71
Stayed overseas for >1 year (%)		57 (18.7)	26 (32.5)	31 (13.8)	<0.001
Stayed in South, East or Southeast Asia for >1 year (%)		40 (13.1)	18 (22.5)	22 (9.8)	0.01
Travelled in the past >1 year (%)		178 (58.4)	47 (58.8)	131 (58.2)	1.0
Travelled in South, East or Southeast Asia in the past 1 year (%)		163 (53.4)	43 (53.8)	120 (53.3)	1.0
Any chronic illnesses (%)		127 (41.6)	33 (41.2)	94 (41.8)	1.0
Hospitalisation in the past 1 year (%)		43 (14.1)	14 (17.5)	29 (12.9)	0.41
Previous antibiotics intake (%)		282 (92.5)	76 (95.0)	206 (91.6)	0.45
Antibiotics in the last 6 months (%)		85 (27.9)	23 (28.8)	62 (27.6)	0.61
Knowledge score >6 (%)		89 (29.2)	33 (41.3)	56 (24.9)	0.01
No left over antibiotics (%)		211 (69.2)	63 (78.8)	148 (65.8)	0.04

*IQR- interquartile range

Out of the 93 ceftriaxone-resistant isolates, 17 were cefoxitin resistant, suggestive of AmpC β-Lactamase production. Only one *Enterobacter cloacae* complex isolate was resistant to ertapenem and was of intermediate susceptibility to meropenem (Table 3). This *Enterobacter cloacae* complex isolate was not a carbapenemase-producer based

on phenotypic and genotypic tests. Eighty-three (83/93, 89.2%) of these ESBL-PE isolates were *E. coli*. The majority of ESBL-PE remained susceptible to aminoglycosides including gentamicin (80/93, 86.0%) and amikacin (91/93, 97.8%) as well as nitrofurantoin (76/93, 81.7%), while ciprofloxacin (53/93, 57.0%) and Sulfamethoxazole-trimethoprim (32/93, 34.4%) resistance were more common.

Table 3. Antibiotic susceptibility of the ceftriaxone-resistant isolates

	E coli (N=83) N (%)	*Klebsiella* (N=6) N (%)	Others^ (N=4) N (%)	Total (N=93) N (%)
Piperacillin-tazobactam	73 (88.0)	4 (66.7)	1 (25.0)	78 (83.9)
Cefepime	35 (42.4)	3 (50)	2 (50.0)	40 (43.0)
Aztreonam	39 (47.0)	2 (33.3)	1 (25.0)	42 (45.2)
Amikacin	82 (98.8)	5 (83.3)	4 (100)	91 (97.8)
Gentamicin	75 (90.4)	3 (50)	2 (50.0)	80 (86.0)
Nitrofuratoin	73 (88.0)	2 (33.3)	1 (25.0)	76 (81.7)
Sulfamethoxazole-trimethoprim	32 (38.6)	0 (0)	0 (0)	32 (34.4)
Ciprofloxacin	48 (57.8)	4 (66.7)	1 (25.0)	53 (57.0)
Fosfomycin	63 (75.9)	1 (16.7)	0 (0)	64 (68.8)
Ertapenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)
Meropenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)

^ Others include *Enterobacter* spp (2), *Proteus mirabillis* (1), *Raoultella ornithinolyitca* (1)

Molecular classification of ESBL-PE

Eighty (80/93, 85%) ESBL-PE isolates from unique participants underwent whole genome sequencing. When two or more isolates grew from a single subject's sample, *E. coli*, the commonest species observed, was selected to facilitate comparisons. Genotypic species determination from the sequence reads correlated completely with the results by MALDI-TOF MS or the Vitek-2 system. Seventy-one (71/80, 88.8%)

isolates were *E. coli*, of which the most common molecular type was sequence type (ST) 131 (11/71, 15.5%) (Table S5). The most frequently observed ESBL gene was CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27 (16/71, 22.5%). *E coli* ST131 were more resistant to fluoroquinolones than non-ST131 isolates (p=0.041). The only significant factor from the questionnaire associated with ESBL-producing *E. coli* ST131 carriage was having more children in the household, but the difference was marginal (mean 0.3±0.7 versus 0.8 ± 1.1, p=0.034).

DISCUSSION

We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy community dwellers in Singapore, twice the rate found in an earlier study in 2014 of patients at an emergency department.[22] Similar rises have been observed globally.[3] Although these figures are lower than the reported prevalence of over 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly regulated antibiotic prescription system similar to Europe and the US where only registered medical practitioners are allowed to prescribe antibiotics, and they must be purchased from licensed dispensers. We did not find any association between fecal carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a city-state and overseas travel is very common, making it hard to detect such a relationship when frequent trips to neighbouring countries are made. However, past residency overseas was strongly associated with colonisation, especially those who lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). Distinctions should be made in future studies on overseas travels and AMR carriage in terms of

duration and frequency of travel, in addition to destinations. The possibility of substantial acquisition of MDRO colonisation and infection through overseas exposure[24,25] once again highlights the urgent need for a regional, collaborative approach to tackling the problem of AMR.

In addition, we did not find an association between recent antibiotic intake in the past 6 months and ESBL-PE carriage. This is inconsistent with previous reports showing that consumption of certain classes of antibiotics such as beta-lactams and fluoroquinolones are risk factors for predispositions to ESBL-PE carriage. [26,27] The possible reasons could be due to the relatively small number of participants who had recent antibiotic intake (85/305, 27.9%), so we were not able to distinguish the specific classes of antibiotics taken by the participants. It is also possible that the dominance of a hyperendemic community-associated clone rather than antibiotic selection pressure alone contributed to this finding.

Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL mechanism, echoing the global dissemination of this hyperendemic clone, especially in the community.[28] Similar reports showed 11.1% (32/287) in China[29] and 4.1% (8/193) in Thailand[30] have been published. The reason for the rapid worldwide expansion and long-term persistence of *E. coli* ST131 is thought to be due to compensatory mutations within the core genome counterbalancing the fitness cost associated with IncF plasmids, thus sustaining its spread even in the absence of direct antibiotic selection pressure.[31] These *E. coli* ST131 are not just prevalent colonisers but have also associated with invasive bloodstream infections in hospitalized patients

in Australia, New Zealand and Singapore.[32] It will be important to better understand the evolutionary ecology and transmission dynamics of this emerging clone.

This study also revealed widespread misconceptions about the utility of antibiotics for viral infections, consistent with the findings of a global survey conducted by the WHO in 2015.[33] We also found that, the public continues to place trust in their primary care doctors and their recommendations. This dependence on physicians is in contrast to doctors' perceptions of patient expectations for antibiotic prescriptions.[34] This discordance has been previously described and is thought to be due to the lack of empowerment of the patient and the erroneous attribution of patient satisfaction to antibiotic prescription rather than a focus on better patient-doctor communication.[35,36]

Engaging and educating both the prescribers and the public may reduce inappropriate antibiotic use,[37,38] and has been identified as a key strategy by the WHO and the UK to tackle AMR.[39,40] One of the most striking findings of this study is that having both the knowledge that antibiotic courses should be completed and not having left over antibiotics is independently associated with the carriage of ESBL-PE. Though these relationships cannot be viewed as causal given the complexities in the emergence and transmission of AMR, there is emerging evidence supporting short course antibiotic therapies, even for severe infections such as bacteremia, given the collateral damage that antibiotics have on host microbiome.[41] The current WHO recommendation remains that full courses of antibiotics should be completed to prevent the onset of resistance.[33] Similar messages are advocated in national

campaigns launched in Australia,[42] the United States[43] and Europe.[44] Given that the minimum effective treatment durations have not been determined for many infections and that a significant proportion of antibiotic prescriptions are inappropriate, the emphasis on completing the course of antibiotics to prevent resistance may have to be re-examined.

To our knowledge, this is the first study that explored antibiotic consumption behavior with the acquisition of MDRO at a community level. This novel approach has the potential to guide clinicians and policy makers in identifying directly actionable interventions for the population. The main weakness of our study is that the questionnaire data is self-reported and subjected to recall and interviewer biases. We minimised these errors by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly. Further research using antibiotic prescription databases can potentially overcome some of the intrinsic biases arising from cross-sectional questionnaires.

CONCLUSION

There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore, especially with *E. coli* ST131 carrying CTX-M. This is correlated with KAP of antibiotic use, especially with the practice of finishing full courses of antibiotics, and prolonged residency in other parts of Asia. Innovative approaches to control AMR that take into account transboundary transmission of resistance and clinical trials to determine the appropriate duration of antimicrobial therapy will be critical to control

the emergence of these resistant clones which have contributed significantly to the current global antibiotic resistance crisis.

CONTRIBUTOR AND GUARANTOR INFORMATION

YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK performed microbiological testing. RTHO planned and conducted genomic sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW performed data analysis. All participated in the writing of the script, and affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained. YM and IS accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and no others meeting the criteria have been omitted.

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COMPETING INTERESTS DECLARATION

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any

organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

DATA SHARING

The authors commit to making the relevant anonymised patient level data available on reasonable request.

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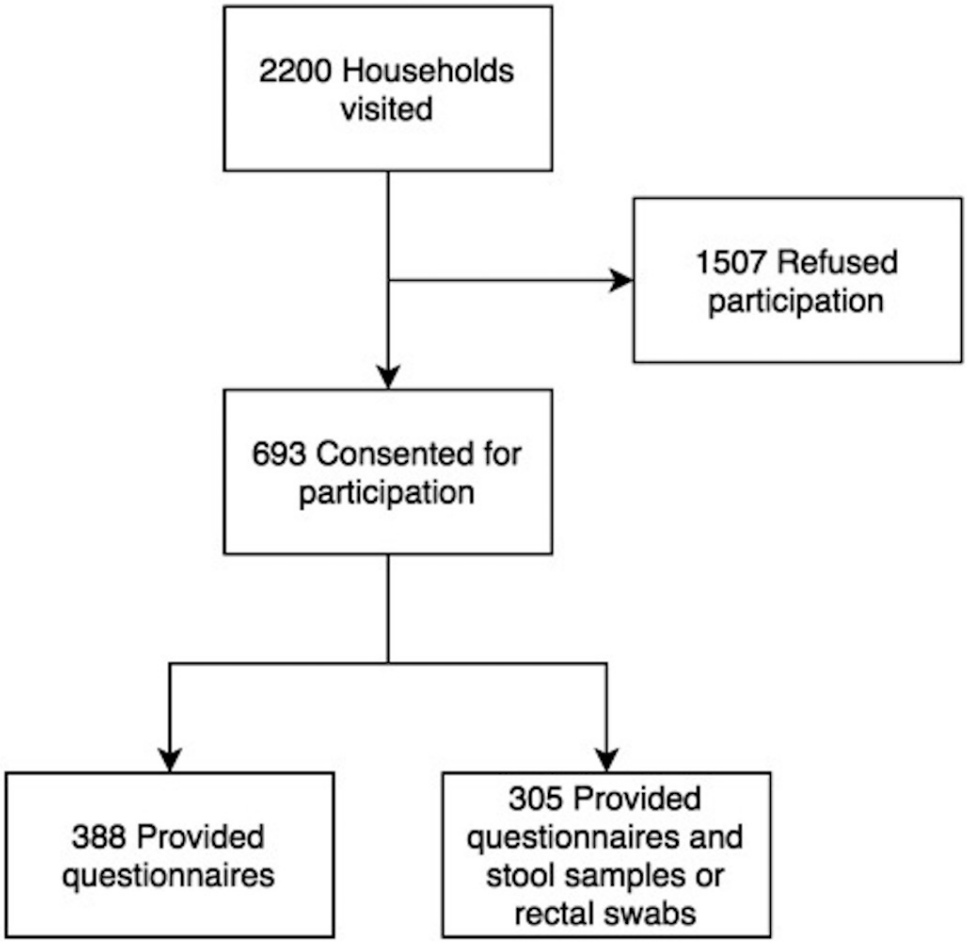

Figure S1. Participant recruitment flowchart

90x87mm (300 x 300 DPI)

Table S1. Study questionnaire

Section 1: Background Data

1. Demographic Data
1.1 Age
1.2 Gender - Male or Female
2. Race - Chinese or Malay or Indian or Others
3. Educational Background
2.1 Highest Education Level Attained- Primary Education or Secondary Education or Tertiary Education or Graduate Education or No formal education
4. Have you ever studied a healthcare-related course? (Medicine, Traditional Chinese Medicine, Therapy, Nursing) - Yes or No
3 Occupation and Financial Status
5. Ocupation:
4 Accommodation
4.1 Housing type- Public housing (1-Room or 2-Room or 3-Room or 4-Room or 5-Room or Executive Apartment) or Landed property
4.2 How many occupants are there living in your house? (including you) Number of Occupants:
4.3.1 How many people in the household are in the following age group? Less than 12 years old:
4.3.2 How many people in the household are in the following age group? More than 65 years old:
6. Do you currently have any dogs or cats at home? - Yes or No
5 Travel history

5.3 Have you travelled to the following places within the past 6 months? – Yes or No

5.3.1 If yes, which of the following places have you been to? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

5.4 Have you lived anywhere else for more than 1 year? – Yes or No

6 If yes, did you live in the following areas? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

7 Medical History

6.1 Do you have any of the following? (You can choose more than one of the following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids, Immunosuppressants etc) and/ or Other medical conditions or None of the above

6.2 When was your last hospitalisation? - Never been hospitalised before or Hospitalised before

6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

6.2.2 How long was your stay? Duration:

6.3 Have you used antibiotics before? - Have never used antibiotics before or Used antibiotics before

6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6

months or More than 6 months ago

Section 2: Assessment of Antibiotic Practices

1. Assessing Health-Seeking and Antibiotic-Seeking Behaviours

Scenario 1: Cough and Runny Nose

1.1.1 Would you go to the doctor for a cough and runny nose that lasted less than 1 week? – Yes or No or I am not sure

1.1.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.1.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

Scenario 2: Diarrhoea and Vomiting

1.2.1 Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than a week? – Yes or No or I am not sure

1.2.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.2.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

2. Assessing Practices of Disposal and Storage of Antibiotics

2.1 What do you usually do with leftover antibiotics? - Usually do not have leftovers

or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the rubbish bin or Others:

3. Assessing Alternative Antibiotic Practices

3.1 Have you ever shared antibiotics with someone else? – Yes or No

3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes or No

Section 3: Attitude Assessment

1. Attitudes Towards Healthcare Provider Prescription

1.1 Sometimes my doctor prioritises what is beneficial for him over my medical needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.3 My doctor is always honest when telling me about all the available treatments for my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

2. Attitudes Towards Potential Educational Interventions

2.1 Which of the following sources of medical information do you trust most? - Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists) or Family and Friends or Online Medical Sources or Television Programmes and Advertisements or Radio Programmes and Advertisements

Section 4: Knowledge Assessment

1. Knowledge on Function of Antibiotics

1.1 Antibiotics are medicines that can treat viral infections. – True or False or I am not sure

1.2 Antibiotics are medicines that can treat bacterial infections. – True or False or I am not sure

1.3 Antibiotics are medicines that can treat fungal infections. – True or False or I am not sure

2. Knowledge on Agents of Infection

2.1 Which of the following most commonly causes running nose and cough? – Virues or Bacteria or I am not sure

2.2 Which of the following most commonly causes diarrhoea? – Virues or Bacteria or I am not sure

3. Knowledge on Proper Use of Antibiotics

3.1 Antibiotics can be obtained at the pharmacist without any prescription. - True or False or I am not sure

3.2 Antibiotics can be stopped when: - You start to feel better or You finish the entire course or You head back to the doctor and he tells you that you can stop

4. Knowledge on Concept of Antibiotic Resistance

4.1 Do you understand what is antibiotic resistance? – Yes or No or I am unsure

4.1.1 If yes, describe what causes antibiotic resistance?

4.2 Which of the following is a consequence of antibiotic resistance? (choose only ONE option) - Antibiotics become more effective at treating infections or Antibiotics become less effective at treating infections or Your body immunity

becomes weaker or Your body immunity becomes stronger

4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am unsure

Table S2. Assessment of knowledge

Questions			N (%) Total N= 693
2.1.1	Antibiotics are medicines that can treat viral infections.	False	149 (21.5%)
		True	335 (48.3%)
		Unsure	209 (30.2%)
2.1.2	Antibiotics are medicines that can treat bacterial infections.	True	419 (60.5%)
		False	50 (7.2%)
		Unsure	224 (32.3%)
2.1.3	Antibiotics are medicines that can treat fungal infections.	False	157 (22.7%)
		True	194 (28.0%)
		Unsure	342 (49.4%)
2.1.4	Which of the following most commonly causes running nose and cough.	Viruses	352 (50.8%)
		Bacteria	130 (18.8%)
		Unsure	211 (30.4%)
2.1.5	Which of the following most commonly causes diarrhoea?	Viruses	98 (14.1%)
		Bacteria	385 (55.6%)
		Unsure	210 (30.3%)
2.1.6	Antibiotics can be stopped when…	You finish the entire course	554 (79.9%)
		When you feel better	95 (13.7%)
		Consult the doctor	44 (6.3%)
2.1.7	Antibiotics can be obtained at the pharmacist without any prescription.	False	564 (81.4%)
		True	29 (4.2%)
		Unsure	100 (14.4%)

2.1.8	What causes antimicrobial resistance? (Open ended)	Inappropriate use of antibiotics	121 (17.5%)
		Wrong or unsure	572 (82.5%)
2.1.9	Which of the following is a consequence of antibiotic resistance?	Antibiotics becoming more effective at treating infections	280 (40.4%)
		Antibiotics becoming less effective at treating infections	111 (16.0%)
		Your body immunity becomes weaker	235 (33.9%)
		Your body immunity becomes stronger	67 (9.7%)
2.1.10	Antibiotic resistance is not present in Singapore yet.	False	255 (36.8%)
		True	77 (11.1%)
		Unsure	361 (52.1%)

Table S3. Assessment of attitude toward primary care

Questions			N (%) N= 693
2.2.1	Sometimes my doctor prioritises what is beneficial for him over my medical needs	Strongly agree	14 (2.0)
		Agree	109 (15.7)
		Neutral	145 (20.9)
		Disagree	335 (48.3)
		Strongly disagree	90 (13.0)
2.2.2	My doctor's medical skills are not as good as they should be	Strongly agree	10 (1.4)
		Agree	83 (12.0)
		Neutral	150 (21.6)
		Disagree	373 (53.8)
		Strongly disagree	77 (11.1)
2.2.3	My doctor is always honest when telling me about all the available treatments for my condition	Strongly agree	100 (14.4)
		Agree	427 (61.6)
		Neutral	115 (16.6)
		Disagree	45 (6.5)
		Strongly disagree	6 (0.9)
2.2.4	I have no worries about putting my life in my doctor's hands	Strongly agree	110 (15.9)
		Agree	363 (52.4)
		Neutral	135 (19.5)
		Disagree	74 (10.7)
		Strongly disagree	11 (1.6)
2.2.5	Which of the following sources of medical information do you trust most?	Healthcare professional's advice	627 (90.6)
		Family and friends	36 (5.2)
		Online medical sources	24 (3.5)

		Television programmes and advertisements	4 (0.6)
		Radio programmes and advertisements	1 (0.1)

Table S4. Assessment of practices

Questions			N (%) Total N= 693
2.3.1.1	Would you go to the doctor for a cough and runny nose that lasted less than 1 week	Yes	294 (42.4)
		No	377 (54.4)
		Unsure	22 (3.2)
2.3.1.2	Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than 1 week?	Yes	414 (59.7)
		No	262 (37.8)
		Unsure	17 (2.5)
2.3.2.1	Would you expect the doctor to prescribe antibiotics for cough and runny nose that lasted less than 1 week to help with the recovery?	Yes	136 (19.6)
		No	508 (73.3)
		Unsure	49 (7.1)
2.3.2.2	Would you expect the doctor to prescribe antibiotics for diarrhoea, vomiting and stomach pain that lasted less than 1 week to help with the recovery?	Yes	120 (17.3)
		No	501 (72.3)
		Unsure	72 (10.4)
2.3.3.1	If the doctor you were seeing does not prescribe you antibiotics for cough and runny nose that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	37 (5.3)
		No	619 (89.3)
		Unsure	37 (5.3)
2.3.3.2	If the doctor you were seeing does not prescribe you antibiotics for diarrhea vomiting and stomach pain that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	40 (5.8)
		No	615 (88.7)
		Unsure	38 (5.5)
2.3.4.1	What do you usually do with left over antibiotics?	No left overs	476 (68.7)
		Disposal in rubbish bin	130 (18.8)
		Keep for future use	60 (8.7)

		Unsure	19 (2.7)
		Pour down sink or toilet bowl	8 (1.2)
2.3.4.2	Have you ever shared antibiotics with anyone else?	Yes	23 (3.3)
		No	670 (94.5)
2.3.4.3	Have you ever taken leftover antibiotics from a previous course of illness?	Yes	38 (5.5)
		No	655 (9.5)

Table S5. Molecular classification of ceftriaxone-resistant E coli isolates

		E coli N=71 (%)		p-value
		ST131 N=11 (%)	Non ST131 N=60 (%)	
Number of resistant genes (mean ± sd)		1.2 ± 0.4	1.9 ± 0.8	0.0012
ESBL genes				
CTXM	15	4 (36.4)	17 (28.3)	0.72
	27	7 (63.6)	9 (15.0)	
	14	0 (0.0)	10 (16.7)	
	55	0 (0.0)	9 (15.0)	
	8	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	9 (15.0)	
	None	0 (0.0)	3 (5.0)	
SHV	12	0 (0.0)	3 (5.0)	1.0
	None	11 (100.0)	57 (95.0)	
TEM	206	1 (9.1)	11 (18.3)	0.11
	198	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	15 (25.0)	

	None	10 (90.9)	31 (51.7)	
OXA		1 (9.1)	3 (5.0)	1.0
Quinolone resistance		8 (72.7)	21 (35.0)	0.041

* Non-ST131 sequence types are: 38 (N=8), 1193 (N=5), 10 (N=4), 48 (N=3), other (N=35), none (N=5)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-8
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	4-5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Supplementary material
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	9-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-15
		(*b*) Report category boundaries when continuous variables were categorized	9-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19-20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Relating knowledge, attitude and practice of antibiotic use to extended spectrum beta-Lactamase producing Enterobacteriaceae carriage – results of a cross-sectional community survey

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Relating knowledge, attitude and practice of antibiotic use to extended spectrum beta-lactamase producing *Enterobacteriaceae* carriage – results of a cross-sectional community Survey

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ABSTRACT

Objectives: To study the correlation between knowledge, attitude and practices (KAP) of antibiotic consumption with epidemiology and molecular characteristics of ESBL-producing *Enterobacteriaceae* (ESBL-PE) carriage, in order to identify modifiable factors and public health interventions to reduce prevalence of multidrug resistant organism (MDRO) colonisation in the community.

Design: Cross-sectional questionnaire of KAP towards antibiotic use and collection of stool samples or rectal swabs. ESBL-PE isolates obtained underwent whole genome sequencing to identify resistance genes.

Setting: A densely populated community in Singapore

Participants: There were 693 healthy community- dwelling questionnaire respondents. Out of which, 305 provided stool samples or rectal swabs.

Results: The overall knowledge of antibiotic use was poor (mean score 4.6/10, IQR 3.0-6.0). 80 participants (80/305, 26.2%) carried at least one ESBL-PE isolate. The most common ESBL-PE was *E. coli* sequence type 131 carrying CTX-M type beta-lactamases (11/71, 15.5%). Living overseas for more than 1 year (OR 3.3, 95% CI 1.6 to 6.9) but not short-term travel, recent hospitalisation or antibiotic intake was associated with ESBL-PE carriage. Interestingly, higher knowledge scores (OR 2.0, 95%CI 1.03 to 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.2 to 4.9) were independent factors associated with ESBL-PE carriage in the multivariate logistic regression model.

Conclusions: While the role of trans-border transmission of antimicrobial resistance is well known, we may have to examine the current recommendation that all antibiotics courses have to be completed. Clinical trials to determine the optimum duration of treatment for common infections are critically important.

(246 words)

ARTICLE SUMMARY

Strengths and limitations of this study

- We adopted a novel approach of correlating the antibiotic consumption behavior of the patients and general public with asymptomatic multidrug resistance colonisation.
- Based on individual-level data, we found a high prevalence of asymptomatic carriage of extended spectrum beta-lactamase producing *Enterobacteriaceae* in a country with strict antibiotic prescription policies, and this was independently associated with not having left over antibiotics.
- We minimised recall and interviewer biases by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly.
- Given that the minimum effective treatment durations for many infections have not been determined and that a significant proportion of antibiotic prescriptions are inappropriate, the widely accepted message on the necessity to complete antibiotic courses to reduce antibiotic resistance may have to be re-examined.
- Our findings have the potential to guide clinicians and policy makers in addressing this question as part of the effort to control antimicrobial resistance.

INTRODUCTION

Multidrug resistant *Enterobacteriaceae* (MDRE) have been identified as "critical priority" resistant organisms by the World Health Organization (WHO) in 2017, and are associated with a high overall all-cause mortality, transmissibility and burden.[1] Resistance in *Enterobacteriaceae* is most commonly mediated via the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases.[2] MDRE infections are difficult to treat with few effective antimicrobials on the horizon.[1] Healthy members of the community are increasingly identified as a reservoir of antimicrobial resistance (AMR), especially in the case of ESBL-producing *Enterobacteriaceae* (ESBL-PE).[3] Asymptomatic carriage of ESBL-PE has been associated with more infections, longer hospitalisations, earlier time to death, and higher hospital costs.[4,5]

South East Asian (SEA) countries are known to be a hot spot for AMR.[6] However, the region is heterogeneous with varying healthcare standards and antimicrobial stewardship and utilisation policies.[7] To aid in designing effective public health policies and engage the community in the campaign against AMR, it is crucial to understand the local knowledge, attitude and practices of antibiotic use. This study aims to correlate the epidemiological and behavioral risk factors of ESBL-PE carriage in Singapore, a high-income country in SEA, as well as delineate the genetic mechanisms associated with these resistant organisms.

METHODOLOGY

Study population

The study was carried out in Clementi Township, a densely populated residential area in the west of Singapore. It comprises 27,142 households with 91,630 residents who are socio-demographically comparable to the general Singapore population in terms of age, gender, ethnicity and housing distribution.[8] The study team returned to non-responding households for up to three times on separate days to maximise the response rate. The first adult above 21 years old in each household who responded to the study team was invited to participate in this cross-sectional study; all consenting individuals undertook a questionnaire, while some additionally consented to provide a rectal swab or stool sample. To calculate the number of samples required to estimate the prevalence of ESBL-PE in the community, we used one-sample Z-test with an estimated prevalence of 50%, a confidence interval of 95% and maximum tolerable error of 10%. This yielded about 100 stool samples. Ethical approval was obtained from National University of Singapore Institutional Review Board (Reference number B-16-245).

Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake and health-seeking behaviour

We conducted a questionnaire study to assess the KAP of participants towards antibiotic use. A 40-item questionnaire was developed after performing a thorough literature review of comparable studies.[9–14] This was then validated by a pilot study involving 75 community-dwelling volunteers to ensure fluency and accuracy in question design and language. A team of thirty-three investigators was trained to administer the survey face-to-face, in languages that the participants are fluent in with standardised explanations, to ensure consistency.

The questionnaire comprised four main sections. The first covered socio-demographic data and recent antibiotic intake. The second was an assessment of antibiotic consumption practices, in which two hypothetical scenarios of diarrhoea and upper respiratory tract symptoms were presented, and participants were asked if they would visit the doctor should they experience these symptoms for less than 1 week, if they would expect or insist on an antibiotic prescription from the doctor's visit, and if they would seek a second opinion if antibiotics were not prescribed. The third component assessed participants' attitudes and trust towards primary care healthcare providers, and was adapted from a validated questionnaire from Hall *et al*.[15] The last component examined participants' knowledge on AMR. The full questionnaire and grading system can be found in Table S1.

Bacterial isolation and antibiotic susceptibility testing

The study team requested fresh stool samples or rectal swabs from all study participants. The samples of those who consented were collected from the participants within 24 hours of production and stored centrally at 0-4°C prior to microbiological processing. All sample processing was carried out in the Singapore General Hospital Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagar™ ESBL* and *CHROMID® CARBA SMART (bioMerieux)* media to detect cephalosporin-resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and used for subsequent species identification and antibiotic susceptibility testing. Species identification was done by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 *(bioMerieux)* system.

Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, cefoxitin, cefepime, amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin, nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin, ertapenem and meropenem were assessed by the disc diffusion method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth generation cephalosporins were identified as potential ESBL producers, while those not susceptible to any carbapenem were identified as potential carbapenemase producers. Potential carbapenemase producers were tested phenotypically for carbapeneasme production by modified Hodge test and KPC/MBL and OXA-48 Confirm Kit (ROSCO). All potential carbapenease producers were also subjected to the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM carbapemase gene sequences.

Whole genome sequencing of ESBL-producing *Enterobacteriaceae*

DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially ESBL- or carbapenemase- producers, with sequencing libraries for each isolate prepared as per manufacturer's recommendation to be multiplexed sequenced on the Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs, having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads was performed using the SPAdes Genome Assembler.[17] Bacterial species were identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence types (MLSTs) were determined by a customized script utilising BLAST search for identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial

resistance owing to the existence of specific gene sequences were performed using SRST2.[20]

Statistical Analysis

Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-PE carriage status and presence of specific resistance genes. Dichotomous variables are expressed in frequencies and percentages, while continuous variables are in means with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in multivariate analyses to identify statistically significant factors that influence and determine KAP and ESBL-PE carriage. Covariates that were found to be statistically significant in the univariate analyses were included in the multivariate models. All tests of significance are performed at α=5%. Statistical analysis was carried out using R Version 1.1.383.[21]

Patient and Public Involvement

A group of 75 community dwellers partnered with us for the design and validation of the study questionnaire to ensure clarity and accuracy, production of informational material to support recruitment, and evaluation of the burden of the sample collection from the patient's perspective. Because there was no clear preference for the sample collection methodology, the study team decided to offer both options of rectal swab and stool collection to the study participants.

RESULTS

From June 2016 to April 2017, we randomly selected 2,200 households in Clementi for home visits. Out of these 2200 households, 693 (31.5%) agreed to participate, of whom 305 (44.0%) also provided stool samples or rectal swabs (Figure S1). Participant demographics are presented in Table 1. The median age of participants was 53 (IQR 38-66). A slight majority were women (393/693, 56.7%). The ethnic distribution of the participants was similar to the wider Singapore population, with 513 (74.0%) Chinese, 78 (11.3%) Malay, and 83 (12.0%) Indian. The majority had received at least secondary school education (534/693, 77.0%), and stayed in public housing apartments (661/693, 95.4%). The median number of occupants per household was 3 (IQR 2-4) persons. A quarter (25.3%, 175/693) reported having taken antibiotics in the past 6 months, and 102 (14.7%) had recently been hospitalised in the past 1 year.

Table 1. Demographics, medical background and antibiotic use of study participants

Characteristic		N (%) Total N=693
Age (median, IQR*)		53.0 (38.0-66.0)
Females		393 (56.7)
Race	Chinese	513 (74.0)
	Malay	78 (11.3)
	Indian	83 (12.0)
	Other ethnicities	19 (2.7)
Education level	Graduate	88 (12.7)
	Diploma	251 (36.2)
	Secondary	195 (28.1)
	Primary	122 (17.6)
	No Formal Education	37 (5.3)
Housing type	1-, 2 or 3-room public housing	334 (48.2)
	4 or 5- room public housing	327 (47.2)
	Private landed property	32 (4.6)
Number of occupants in the household	Overall (median, IQR)	3 (2-4)
	≤ 3 persons	369 (53.2)
	4-5 persons	257 (37.1)
	≥ 6 persons	67 (9.7)

Comorbidities	Any chronic illnesses	239 (34.5)
	Hypertension	105 (15.2)
	Hyperlipidemia	76 (11.0)
	Diabetes mellitus	67 (9.7)
Recent hospitalisation in the past 1 year		102 (14.7)
Antibiotic consumption	Within past 6 months	175 (25.3)
	More than 6 months ago	441 (63.6)
	Never taken antibiotics	77 (11.1)

*IQR- interquartile range, ^Immunocompromised – Use of chemotherapy, corticosteroids or immunosuppressants in the past 6 months

The survey revealed widespread misinformation about antibiotics, with a mean knowledge score of only 4.6 (IQR 3.0-6.0) out of 10 (Table S2). Although the majority of participants knew that viruses are the most common cause of upper respiratory tract infections, a significant proportion (335/693, 48.3%) believed that antibiotics could be used for viral infections and 385 (385/693, 55.6%) thought that the most common cause of diarrhoea was bacteria. The questionnaire also explored participants' compliance to the widely accepted view of completing antibiotic courses. The majority (554/693, 79.9%) said they would complete the course of antibiotics prescribed, while 13.7% (95/693) would stop taking antibiotics when they start to feel better, and 6.3% (44/693) preferred to seek the doctor's opinion before stopping the course. Most participants (564/693, 81.4%) were aware that antibiotics are prescription-only drugs in Singapore, but were unable to correctly answer questions related to AMR, with 82.5% (572/693) not knowing what causes AMR, and 63.2% (438/693) believing AMR was not present in Singapore. Level of education ($p<0.001$) and staying in larger housing ($p=0.037$)—the usual proxies for socio-economic status in Singapore—were independent factors associated with higher total knowledge scores. However, higher knowledge scores were not strongly related to participants' trust in primary care physicians (OR 1.08, 95%CI 0.97-1.20) or the

expectation of an antibiotic prescription for common viral infections (OR 0.98, 95%CI 0.96-1.0).

A large majority of the community continued to place trust in their primary care doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted healthcare professionals as their primary source of medical information, over the Internet, media and family and friends. There were no significant associations between demographic factors and attitude scores in contrast to the differences seen in knowledge scores.

In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and vomiting), although about half of the participants (294/693, 42.4% for cough and runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the doctor for common complaints lasting less than 1 week, only 18.5% (average 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not prescribed during the initial visit, very few (average 39/693, 5.6%) reported they would insist on antibiotic prescription or seek a second opinion. The only independent factor associated with the expectation of an antibiotic prescription was younger age (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3% (23/693) admitted to having previously shared antibiotics with family members and 5.5% (38/693) to having taken leftover antibiotics from a previous illness.

Asymptomatic carriage of ESBL-PE

Three hundred and five participants (305/693, 44.0%) provided rectal swabs or stool samples for microbiology cultures. The participants who provided stool samples were not significantly different from those who did not, in terms of age, gender and education level. Eighty participants (80/305, 26.2%, 95%CI: 21.5-31.6%) were found to carry at least one ceftriaxone non-susceptible *Enterobacteriaceae* isolate. One hundred and fifteen isolates were detected on the ESBL screening media, of which 93 were ceftriaxone resistant or intermediate *Enterobacteriaceae*. Six bacterial isolates were detected on the CRE screening media, none of which were confirmed to be carbapenemase-producing *Enterobacteriaceae*. The factors associated with ESBL-PE carriage from multivariate logistic regression analysis were residency overseas for more than 1 year (OR 3.3, 95%CI 1.6-6.9), with the most common location being other parts of Asia, scoring higher than 6 on the knowledge component in the questionnaire (OR 2.0 95%CI 1.03- 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.24-4.9). Interestingly, recent hospitalisation and reported antibiotic intake were not associated with ESBL-PE carriage (Table 2).

Table 2. Univariate analysis of demographic characteristics associated with carriage of ceftriaxone- resistant *Enterobactriaceae*

Factors		Total N=305	Carriers N=80	Non-carriers N=225	p-values
Age (median, IQR*)		54.0 (41.0-65.0)	56.0 (38.8-66.0)	54.0 (41.0-65.0)	0.79
Females (%)		169 (55.4)	46 (57.5)	123 (54.7)	0.76
Ethnicity (%)	Chinese	237 (77.7)	67 (83.8)	170 (75.6)	0.24
	Malay	28 (9.2)	3 (3.8)	25 (11.1)	
	Indian	30 (9.8)	7 (8.8)	23 (10.2)	
	Others	10 (3.3)	3 (3.8)	7 (3.1)	
Education (%)	No formal	11 (3.6)	4 (5.0)	7 (3.1)	0.45

	education				
	Primary	57 (18.7)	12 (15.0)	45 (20.0)	
	Secondary	93 (30.5)	21 (26.2)	72 (32.0)	
	Tertiary	110 (36.1)	31 (38.8)	79 (35.1)	
	Graduate	34 (11.1)	12 (15.0)	22 (9.8)	
Housing (%)	HDB 1- and 2-room	23 (7.5)	5 (6.2)	18 (8.0)	0.75
	HDB 3-room	115 (37.7)	32 (40.0)	83 (36.9)	
	HDB 4-room	98 (32.1)	24 (30.0)	74 (32.9)	
	HDB 5-room and Executive Apartment	47 (15.4)	11 (13.8)	36 (16.0)	
	Landed Property	22 (7.2)	8 (10.0)	14 (6.2)	
Pets (%)		33 (10.8)	7 (8.8)	26 (11.6)	0.75
Number of occupants in the household (mean, sd)		3.6 (1.6)	3.6 (1.6)	3.6 (1.6)	0.71
Stayed overseas for >1 year (%)		57 (18.7)	26 (32.5)	31 (13.8)	<0.001
Stayed in South, East or Southeast Asia for >1 year (%)		40 (13.1)	18 (22.5)	22 (9.8)	0.01
Travelled in the past >1 year (%)		178 (58.4)	47 (58.8)	131 (58.2)	1.0
Travelled in South, East or Southeast Asia in the past 1 year (%)		163 (53.4)	43 (53.8)	120 (53.3)	1.0
Any chronic illnesses (%)		127 (41.6)	33 (41.2)	94 (41.8)	1.0
Hospitalisation in the past 1 year (%)		43 (14.1)	14 (17.5)	29 (12.9)	0.41
Previous antibiotics intake (%)		282 (92.5)	76 (95.0)	206 (91.6)	0.45
Antibiotics in the last 6 months (%)		85 (27.9)	23 (28.8)	62 (27.6)	0.61
Knowledge score >6 (%)		89 (29.2)	33 (41.3)	56 (24.9)	0.01
No left over antibiotics (%)		211 (69.2)	63 (78.8)	148 (65.8)	0.04

*IQR- interquartile range

Out of the 93 ceftriaxone-resistant isolates, 17 were cefoxitin resistant, suggestive of AmpC β-Lactamase production. Only one *Enterobacter cloacae* complex isolate was resistant to ertapenem and was of intermediate susceptibility to meropenem (Table 3). This *Enterobacter cloacae* complex isolate was not a carbapenemase-producer based

on phenotypic and genotypic tests. Eighty-three (83/93, 89.2%) of these ESBL-PE isolates were *E. coli*. The majority of ESBL-PE remained susceptible to aminoglycosides including gentamicin (80/93, 86.0%) and amikacin (91/93, 97.8%) as well as nitrofurantoin (76/93, 81.7%), while ciprofloxacin (53/93, 57.0%) and Sulfamethoxazole-trimethoprim (32/93, 34.4%) resistance were more common.

Table 3. Antibiotic susceptibility of the ceftriaxone-resistant isolates

	E coli (N=83) N (%)	*Klebsiella* (N=6) N (%)	Others^ (N=4) N (%)	Total (N=93) N (%)
Piperacillin-tazobactam	73 (88.0)	4 (66.7)	1 (25.0)	78 (83.9)
Cefepime	35 (42.4)	3 (50)	2 (50.0)	40 (43.0)
Aztreonam	39 (47.0)	2 (33.3)	1 (25.0)	42 (45.2)
Amikacin	82 (98.8)	5 (83.3)	4 (100)	91 (97.8)
Gentamicin	75 (90.4)	3 (50)	2 (50.0)	80 (86.0)
Nitrofuratoin	73 (88.0)	2 (33.3)	1 (25.0)	76 (81.7)
Sulfamethoxazole-trimethoprim	32 (38.6)	0 (0)	0 (0)	32 (34.4)
Ciprofloxacin	48 (57.8)	4 (66.7)	1 (25.0)	53 (57.0)
Fosfomycin	63 (75.9)	1 (16.7)	0 (0)	64 (68.8)
Ertapenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)
Meropenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)

^ Others include *Enterobacter* spp (2), *Proteus mirabillis* (1), *Raoultella ornithinolyitca* (1)

Molecular classification of ESBL-PE

Eighty (80/93, 85%) ESBL-PE isolates from unique participants underwent whole genome sequencing. When two or more isolates grew from a single subject's sample, *E. coli*, the commonest species observed, was selected to facilitate comparisons. Genotypic species determination from the sequence reads correlated completely with the results by MALDI-TOF MS or the Vitek-2 system. Seventy-one (71/80, 88.8%)

isolates were *E. coli*, of which the most common molecular type was sequence type (ST) 131 (11/71, 15.5%) (Table S5). The most frequently observed ESBL gene was CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27 (16/71, 22.5%). *E coli* ST131 were more resistant to fluoroquinolones than non-ST131 isolates (p=0.041). The only significant factor from the questionnaire associated with ESBL-producing *E. coli* ST131 carriage was having more children in the household, but the difference was marginal (mean 0.3±0.7 versus 0.8 ± 1.1, p=0.034).

DISCUSSION

We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy community dwellers in Singapore, twice the rate found in an earlier study in 2014 of patients at an emergency department.[22] Similar rises have been observed globally.[3] Although these figures are lower than the reported prevalence of over 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly regulated antibiotic prescription system similar to Europe and the US where only registered medical practitioners are allowed to prescribe antibiotics, and they must be purchased from licensed dispensers. We did not find any association between fecal carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a city-state and overseas travel is very common, making it hard to detect such a relationship when frequent trips to neighbouring countries are made. However, past residency overseas was strongly associated with colonisation, especially those who lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). Distinctions should be made in future studies on overseas travels and AMR carriage in terms of

duration and frequency of travel, in addition to destinations. The possibility of substantial acquisition of MDRO colonisation and infection through overseas exposure[24,25] once again highlights the urgent need for a regional, collaborative approach to tackling the problem of AMR.

In addition, we did not find an association between recent antibiotic intake in the past 6 months and ESBL-PE carriage. This is inconsistent with previous reports showing that consumption of certain classes of antibiotics such as beta-lactams and fluoroquinolones are risk factors for predispositions to ESBL-PE carriage. [26,27] The possible reasons could be due to the relatively small number of participants who had recent antibiotic intake (85/305, 27.9%), so we were not able to distinguish the specific classes of antibiotics taken by the participants. It is also possible that the dominance of a hyperendemic community-associated clone rather than antibiotic selection pressure alone contributed to this finding.

Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL mechanism, echoing the global dissemination of this hyperendemic clone, especially in the community.[28] Similar reports showed 11.1% (32/287) in China[29] and 4.1% (8/193) in Thailand[30] have been published. The reason for the rapid worldwide expansion and long-term persistence of *E. coli* ST131 is thought to be due to compensatory mutations within the core genome counterbalancing the fitness cost associated with IncF plasmids, thus sustaining its spread even in the absence of direct antibiotic selection pressure.[31] These *E. coli* ST131 are not just prevalent colonisers but have also associated with invasive bloodstream infections in hospitalized patients

in Australia, New Zealand and Singapore.[32] It will be important to better understand the evolutionary ecology and transmission dynamics of this emerging clone.

This study also revealed widespread misconceptions about the utility of antibiotics for viral infections, consistent with the findings of a global survey conducted by the WHO in 2015.[33] We also found that, the public continues to place trust in their primary care doctors and their recommendations. This dependence on physicians is in contrast to doctors' perceptions of patient expectations for antibiotic prescriptions.[34] This discordance has been previously described and is thought to be due to the lack of empowerment of the patient and the erroneous attribution of patient satisfaction to antibiotic prescription rather than a focus on better patient-doctor communication.[35,36]

Engaging and educating both the prescribers and the public may reduce inappropriate antibiotic use,[37,38] and has been identified as a key strategy by the WHO and the UK to tackle AMR.[39,40] One of the most striking findings of this study is that having both the knowledge that antibiotic courses should be completed and not having left over antibiotics is independently associated with the carriage of ESBL-PE. Though these relationships cannot be viewed as causal given the complexities in the emergence and transmission of AMR, there is emerging evidence supporting short course antibiotic therapies, even for severe infections such as bacteremia, given the collateral damage that antibiotics have on host microbiome.[41] The current WHO recommendation remains that full courses of antibiotics should be completed to prevent the onset of resistance.[33] Similar messages are advocated in national

campaigns launched in Australia,[42] the United States[43] and Europe.[44] Given that the minimum effective treatment durations have not been determined for many infections and that a significant proportion of antibiotic prescriptions are inappropriate, the emphasis on completing the course of antibiotics to prevent resistance may have to be re-examined.

To our knowledge, this is the first study that explored antibiotic consumption behavior with the acquisition of MDRO at a community level. This novel approach has the potential to guide clinicians and policy makers in identifying directly actionable interventions for the population. The main weakness of our study is that the questionnaire data is self-reported and subjected to recall and interviewer biases. We minimised these errors by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly. Further research using antibiotic prescription databases can potentially overcome some of the intrinsic biases arising from cross-sectional questionnaires.

CONCLUSION

There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore, especially with *E. coli* ST131 carrying CTX-M. This is correlated with KAP of antibiotic use, especially with the practice of finishing full courses of antibiotics, and prolonged residency in other parts of Asia. Innovative approaches to control AMR that take into account transboundary transmission of resistance and clinical trials to determine the appropriate duration of antimicrobial therapy will be critical to control

the emergence of these resistant clones which have contributed significantly to the current global antibiotic resistance crisis.

CONTRIBUTOR AND GUARANTOR INFORMATION

YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK performed microbiological testing. RTHO planned and conducted genomic sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW performed data analysis. All participated in the writing of the script, and affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained. YM and IS accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and no others meeting the criteria have been omitted.

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COMPETING INTERESTS DECLARATION

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

DATA SHARING

The authors commit to making the relevant anonymised patient level data available on reasonable request.

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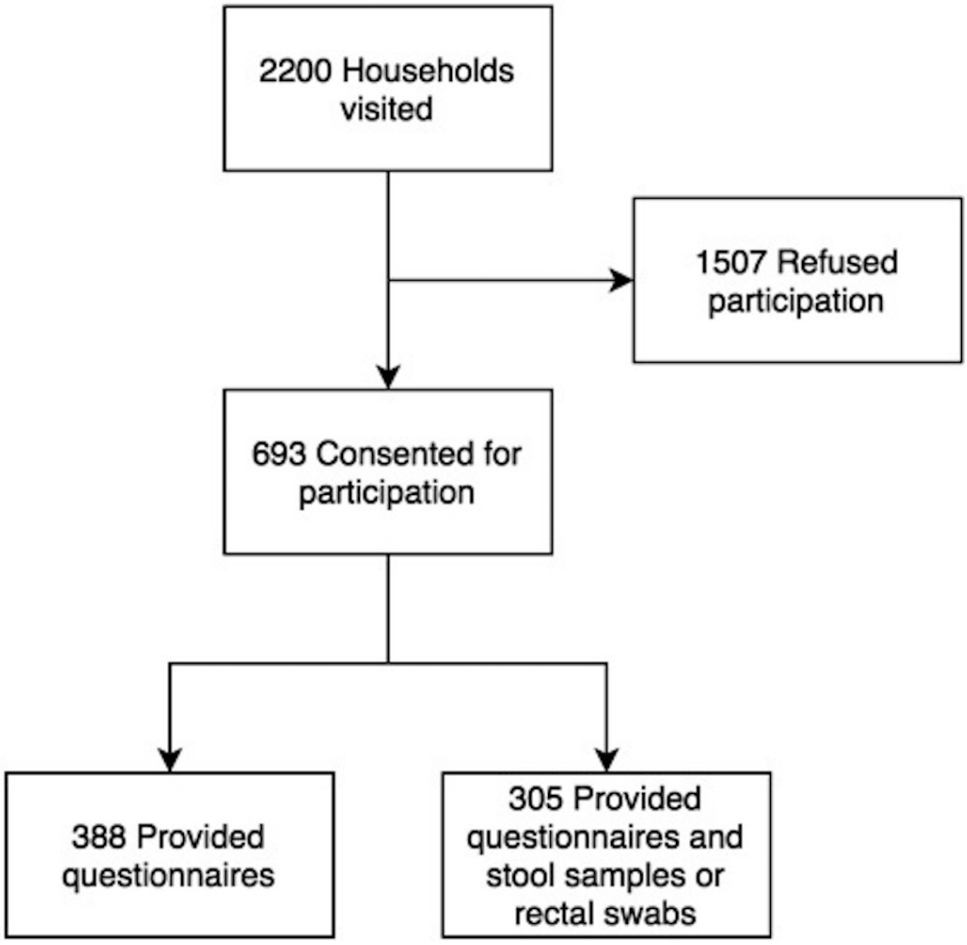

Figure S1. Participant recruitment flowchart

90x87mm (300 x 300 DPI)

Table S1. Study questionnaire

Section 1: Background Data

1. Demographic Data
1.1 Age
1.2 Gender - Male or Female
2. Race - Chinese or Malay or Indian or Others
3. Educational Background
2.1 Highest Education Level Attained- Primary Education or Secondary Education or Tertiary Education or Graduate Education or No formal education
4. Have you ever studied a healthcare-related course? (Medicine, Traditional Chinese Medicine, Therapy, Nursing) - Yes or No
3 Occupation and Financial Status
5. Ocupation:
4 Accommodation
4.1 Housing type- Public housing (1-Room or 2-Room or 3-Room or 4-Room or 5-Room or Executive Apartment) or Landed property
4.2 How many occupants are there living in your house? (including you) Number of Occupants:
4.3.1 How many people in the household are in the following age group? Less than 12 years old:
4.3.2 How many people in the household are in the following age group? More than 65 years old:
6. Do you currently have any dogs or cats at home? - Yes or No
5 Travel history

5.3 Have you travelled to the following places within the past 6 months? – Yes or No

5.3.1 If yes, which of the following places have you been to? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

5.4 Have you lived anywhere else for more than 1 year? – Yes or No

6 If yes, did you live in the following areas? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

7 Medical History

6.1 Do you have any of the following? (You can choose more than one of the following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids, Immunosuppressants etc) and/ or Other medical conditions or None of the above

6.2 When was your last hospitalisation? - Never been hospitalised before or Hospitalised before

6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

6.2.2 How long was your stay? Duration:

6.3 Have you used antibiotics before? - Have never used antibiotics before or Used antibiotics before

6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6

months or More than 6 months ago

Section 2: Assessment of Antibiotic Practices

1. Assessing Health-Seeking and Antibiotic-Seeking Behaviours

Scenario 1: Cough and Runny Nose

1.1.1 Would you go to the doctor for a cough and runny nose that lasted less than 1 week? – Yes or No or I am not sure

1.1.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.1.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

Scenario 2: Diarrhoea and Vomiting

1.2.1 Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than a week? – Yes or No or I am not sure

1.2.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.2.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

2. Assessing Practices of Disposal and Storage of Antibiotics

2.1 What do you usually do with leftover antibiotics? - Usually do not have leftovers

or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the rubbish bin or Others:

3. Assessing Alternative Antibiotic Practices

3.1 Have you ever shared antibiotics with someone else? – Yes or No

3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes or No

Section 3: Attitude Assessment

1. Attitudes Towards Healthcare Provider Prescription

1.1 Sometimes my doctor prioritises what is beneficial for him over my medical needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.3 My doctor is always honest when telling me about all the available treatments for my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

2. Attitudes Towards Potential Educational Interventions

2.1 Which of the following sources of medical information do you trust most? - Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists) or Family and Friends or Online Medical Sources or Television Programmes and Advertisements or Radio Programmes and Advertisements

Section 4: Knowledge Assessment

1. Knowledge on Function of Antibiotics

1.1 Antibiotics are medicines that can treat viral infections. – True or False or I am not sure

1.2 Antibiotics are medicines that can treat bacterial infections. – True or False or I am not sure

1.3 Antibiotics are medicines that can treat fungal infections. – True or False or I am not sure

2. Knowledge on Agents of Infection

2.1 Which of the following most commonly causes running nose and cough? – Virues or Bacteria or I am not sure

2.2 Which of the following most commonly causes diarrhoea? – Virues or Bacteria or I am not sure

3. Knowledge on Proper Use of Antibiotics

3.1 Antibiotics can be obtained at the pharmacist without any prescription. - True or False or I am not sure

3.2 Antibiotics can be stopped when: - You start to feel better or You finish the entire course or You head back to the doctor and he tells you that you can stop

4. Knowledge on Concept of Antibiotic Resistance

4.1 Do you understand what is antibiotic resistance? – Yes or No or I am unsure

4.1.1 If yes, describe what causes antibiotic resistance?

4.2 Which of the following is a consequence of antibiotic resistance? (choose only ONE option) - Antibiotics become more effective at treating infections or Antibiotics become less effective at treating infections or Your body immunity

becomes weaker or Your body immunity becomes stronger

4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am unsure

Table S2. Assessment of knowledge

Questions			N (%) Total N= 693
2.1.1	Antibiotics are medicines that can treat viral infections.	False	149 (21.5%)
		True	335 (48.3%)
		Unsure	209 (30.2%)
2.1.2	Antibiotics are medicines that can treat bacterial infections.	True	419 (60.5%)
		False	50 (7.2%)
		Unsure	224 (32.3%)
2.1.3	Antibiotics are medicines that can treat fungal infections.	False	157 (22.7%)
		True	194 (28.0%)
		Unsure	342 (49.4%)
2.1.4	Which of the following most commonly causes running nose and cough.	Viruses	352 (50.8%)
		Bacteria	130 (18.8%)
		Unsure	211 (30.4%)
2.1.5	Which of the following most commonly causes diarrhoea?	Viruses	98 (14.1%)
		Bacteria	385 (55.6%)
		Unsure	210 (30.3%)
2.1.6	Antibiotics can be stopped when…	You finish the entire course	554 (79.9%)
		When you feel better	95 (13.7%)
		Consult the doctor	44 (6.3%)
2.1.7	Antibiotics can be obtained at the pharmacist without any prescription.	False	564 (81.4%)
		True	29 (4.2%)
		Unsure	100 (14.4%)

2.1.8	What causes antimicrobial resistance? (Open ended)	Inappropriate use of antibiotics	121 (17.5%)
		Wrong or unsure	572 (82.5%)
2.1.9	Which of the following is a consequence of antibiotic resistance?	Antibiotics becoming more effective at treating infections	280 (40.4%)
		Antibiotics becoming less effective at treating infections	111 (16.0%)
		Your body immunity becomes weaker	235 (33.9%)
		Your body immunity becomes stronger	67 (9.7%)
2.1.10	Antibiotic resistance is not present in Singapore yet.	False	255 (36.8%)
		True	77 (11.1%)
		Unsure	361 (52.1%)

Table S3. Assessment of attitude toward primary care

Questions			N (%) N= 693
2.2.1	Sometimes my doctor prioritises what is beneficial for him over my medical needs	Strongly agree	14 (2.0)
		Agree	109 (15.7)
		Neutral	145 (20.9)
		Disagree	335 (48.3)
		Strongly disagree	90 (13.0)
2.2.2	My doctor's medical skills are not as good as they should be	Strongly agree	10 (1.4)
		Agree	83 (12.0)
		Neutral	150 (21.6)
		Disagree	373 (53.8)
		Strongly disagree	77 (11.1)
2.2.3	My doctor is always honest when telling me about all the available treatments for my condition	Strongly agree	100 (14.4)
		Agree	427 (61.6)
		Neutral	115 (16.6)
		Disagree	45 (6.5)
		Strongly disagree	6 (0.9)
2.2.4	I have no worries about putting my life in my doctor's hands	Strongly agree	110 (15.9)
		Agree	363 (52.4)
		Neutral	135 (19.5)
		Disagree	74 (10.7)
		Strongly disagree	11 (1.6)
2.2.5	Which of the following sources of medical information do you trust most?	Healthcare professional's advice	627 (90.6)
		Family and friends	36 (5.2)
		Online medical sources	24 (3.5)

		Television programmes and advertisements	4 (0.6)
		Radio programmes and advertisements	1 (0.1)

Table S4. Assessment of practices

Questions			N (%) Total N= 693
2.3.1.1	Would you go to the doctor for a cough and runny nose that lasted less than 1 week	Yes	294 (42.4)
		No	377 (54.4)
		Unsure	22 (3.2)
2.3.1.2	Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than 1 week?	Yes	414 (59.7)
		No	262 (37.8)
		Unsure	17 (2.5)
2.3.2.1	Would you expect the doctor to prescribe antibiotics for cough and runny nose that lasted less than 1 week to help with the recovery?	Yes	136 (19.6)
		No	508 (73.3)
		Unsure	49 (7.1)
2.3.2.2	Would you expect the doctor to prescribe antibiotics for diarrhoea, vomiting and stomach pain that lasted less than 1 week to help with the recovery?	Yes	120 (17.3)
		No	501 (72.3)
		Unsure	72 (10.4)
2.3.3.1	If the doctor you were seeing does not prescribe you antibiotics for cough and runny nose that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	37 (5.3)
		No	619 (89.3)
		Unsure	37 (5.3)
2.3.3.2	If the doctor you were seeing does not prescribe you antibiotics for diarrhea vomiting and stomach pain that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	40 (5.8)
		No	615 (88.7)
		Unsure	38 (5.5)
2.3.4.1	What do you usually do with left over antibiotics?	No left overs	476 (68.7)
		Disposal in rubbish bin	130 (18.8)
		Keep for future use	60 (8.7)

		Unsure	19 (2.7)
		Pour down sink or toilet bowl	8 (1.2)
2.3.4.2	Have you ever shared antibiotics with anyone else?	Yes	23 (3.3)
		No	670 (94.5)
2.3.4.3	Have you ever taken leftover antibiotics from a previous course of illness?	Yes	38 (5.5)
		No	655 (9.5)

Table S5. Molecular classification of ceftriaxone-resistant E coli isolates

		E coli N=71 (%)		p-value
		ST131 N=11 (%)	Non ST131 N=60 (%)	
Number of resistant genes (mean ± sd)		1.2 ± 0.4	1.9 ± 0.8	0.0012
ESBL genes				
CTXM	15	4 (36.4)	17 (28.3)	0.72
	27	7 (63.6)	9 (15.0)	
	14	0 (0.0)	10 (16.7)	
	55	0 (0.0)	9 (15.0)	
	8	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	9 (15.0)	
	None	0 (0.0)	3 (5.0)	
SHV	12	0 (0.0)	3 (5.0)	1.0
	None	11 (100.0)	57 (95.0)	
TEM	206	1 (9.1)	11 (18.3)	0.11
	198	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	15 (25.0)	

	None	10 (90.9)	31 (51.7)	
OXA		1 (9.1)	3 (5.0)	1.0
Quinolone resistance		8 (72.7)	21 (35.0)	0.041

* Non-ST131 sequence types are: 38 (N=8), 1193 (N=5), 10 (N=4), 48 (N=3), other (N=35), none (N=5)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-8
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	4-5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Supplementary material
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	9-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-15
		(*b*) Report category boundaries when continuous variables were categorized	9-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19-20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Relating knowledge, attitude and practice of antibiotic use to extended spectrum beta-Lactamase producing Enterobacteriaceae carriage – results of a cross-sectional community survey

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Relating knowledge, attitude and practice of antibiotic use to extended spectrum beta-lactamase producing *Enterobacteriaceae* carriage – results of a cross-sectional community Survey

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ABSTRACT

Objectives: To study the correlation between knowledge, attitude and practices (KAP) of antibiotic consumption with epidemiology and molecular characteristics of ESBL-producing *Enterobacteriaceae* (ESBL-PE) carriage, in order to identify modifiable factors and public health interventions to reduce prevalence of multidrug resistant organism (MDRO) colonisation in the community.

Design: Cross-sectional questionnaire of KAP towards antibiotic use and collection of stool samples or rectal swabs. ESBL-PE isolates obtained underwent whole genome sequencing to identify resistance genes.

Setting: A densely populated community in Singapore

Participants: There were 693 healthy community- dwelling questionnaire respondents. Out of which, 305 provided stool samples or rectal swabs.

Results: The overall knowledge of antibiotic use was poor (mean score 4.6/10, IQR 3.0-6.0). 80 participants (80/305, 26.2%) carried at least one ESBL-PE isolate. The most common ESBL-PE was *E. coli* sequence type 131 carrying CTX-M type beta-lactamases (11/71, 15.5%). Living overseas for more than 1 year (OR 3.3, 95% CI 1.6 to 6.9) but not short-term travel, recent hospitalisation or antibiotic intake was associated with ESBL-PE carriage. Interestingly, higher knowledge scores (OR 2.0, 95%CI 1.03 to 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.2 to 4.9) were independent factors associated with ESBL-PE carriage in the multivariate logistic regression model.

Conclusions: While the role of trans-border transmission of antimicrobial resistance is well known, we may have to examine the current recommendation that all antibiotics courses have to be completed. Clinical trials to determine the optimum duration of treatment for common infections are critically important.

(246 words)

ARTICLE SUMMARY

Strengths and limitations of this study

- Based on individual-level data, we adopted a novel approach of correlating knowledge, attitude and practice of antibiotic use with asymptomatic carriage of extended spectrum beta-lactamase producing *Enterobacteriaceae* to identify modifiable factors to mitigate antimicrobial resistance in the community.
- We randomly sampled a large number of households in the community representative of the Singaporean general public in terms of demographics and socioeconomic status.
- Extended spectrum beta-lactamase producing *Enterobacteriaceae* were confirmed with both phenotypic antibiotic susceptibilities and whole genome sequencing.
- We minimised recall and interviewer biases by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly.
- Correlations found in the study cannot be viewed as causal given the complexities in the emergence and transmission of AMR.

INTRODUCTION

Multidrug resistant *Enterobacteriaceae* (MDRE) have been identified as "critical priority" resistant organisms by the World Health Organization (WHO) in 2017, and are associated with a high overall all-cause mortality, transmissibility and burden.[1] Resistance in *Enterobacteriaceae* is most commonly mediated via the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases.[2] MDRE infections are difficult to treat with few effective antimicrobials on the horizon.[1] Healthy members of the community are increasingly identified as a reservoir of antimicrobial resistance (AMR), especially in the case of ESBL-producing *Enterobacteriaceae* (ESBL-PE).[3] Asymptomatic carriage of ESBL-PE has been associated with more infections, longer hospitalisations, earlier time to death, and higher hospital costs.[4,5]

South East Asian (SEA) countries are known to be a hot spot for AMR.[6] However, the region is heterogeneous with varying healthcare standards and antimicrobial stewardship and utilisation policies.[7] To aid in designing effective public health policies and engage the community in the campaign against AMR, it is crucial to understand the local knowledge, attitude and practices of antibiotic use. This study aims to correlate the epidemiological and behavioral risk factors of ESBL-PE carriage in Singapore, a high-income country in SEA, as well as delineate the genetic mechanisms associated with these resistant organisms.

METHODOLOGY

Study population

The study was carried out in Clementi Township, a densely populated residential area in the west of Singapore. It comprises 27,142 households with 91,630 residents who are socio-demographically comparable to the general Singapore population in terms of age, gender, ethnicity and housing distribution.[8] The study team returned to non-responding households for up to three times on separate days to maximise the response rate. The first adult above 21 years old in each household who responded to the study team was invited to participate in this cross-sectional study; all consenting individuals undertook a questionnaire, while some additionally consented to provide a rectal swab or stool sample. To calculate the number of samples required to estimate the prevalence of ESBL-PE in the community, we used one-sample Z-test with an estimated prevalence of 50%, a confidence interval of 95% and maximum tolerable error of 10%. This yielded about 100 stool samples. Ethical approval was obtained from National University of Singapore Institutional Review Board (Reference number B-16-245).

Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake and health-seeking behaviour

We conducted a questionnaire study to assess the KAP of participants towards antibiotic use. A 40-item questionnaire was developed after performing a thorough literature review of comparable studies.[9–14] This was then validated by a pilot study involving 75 community-dwelling volunteers to ensure fluency and accuracy in question design and language. A team of thirty-three investigators was trained to administer the survey face-to-face, in languages that the participants are fluent in with standardised explanations, to ensure consistency.

The questionnaire comprised four main sections. The first covered socio-demographic data and recent antibiotic intake. The second was an assessment of antibiotic consumption practices, in which two hypothetical scenarios of diarrhoea and upper respiratory tract symptoms were presented, and participants were asked if they would visit the doctor should they experience these symptoms for less than 1 week, if they would expect or insist on an antibiotic prescription from the doctor's visit, and if they would seek a second opinion if antibiotics were not prescribed. The third component assessed participants' attitudes and trust towards primary care healthcare providers, and was adapted from a validated questionnaire from Hall *et al*.[15] The last component examined participants' knowledge on AMR. The full questionnaire and grading system can be found in Table S1.

Bacterial isolation and antibiotic susceptibility testing

The study team requested fresh stool samples or rectal swabs from all study participants. The samples of those who consented were collected from the participants within 24 hours of production and stored centrally at 0-4°C prior to microbiological processing. All sample processing was carried out in the Singapore General Hospital Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagar™ ESBL* and *CHROMID® CARBA SMART (bioMerieux)* media to detect cephalosporin-resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and used for subsequent species identification and antibiotic susceptibility testing. Species identification was done by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 *(bioMerieux)* system.

Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, cefoxitin, cefepime, amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin, nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin, ertapenem and meropenem were assessed by the disc diffusion method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth generation cephalosporins were identified as potential ESBL producers, while those not susceptible to any carbapenem were identified as potential carbapenemase producers. Potential carbapenemase producers were tested phenotypically for carbapeneasme production by modified Hodge test and KPC/MBL and OXA-48 Confirm Kit (ROSCO). All potential carbapenease producers were also subjected to the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM carbapemase gene sequences.

Whole genome sequencing of ESBL-producing *Enterobacteriaceae*

DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially ESBL- or carbapenemase- producers, with sequencing libraries for each isolate prepared as per manufacturer's recommendation to be multiplexed sequenced on the Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs, having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads was performed using the SPAdes Genome Assembler.[17] Bacterial species were identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence types (MLSTs) were determined by a customized script utilising BLAST search for identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial

resistance owing to the existence of specific gene sequences were performed using SRST2.[20]

Statistical Analysis

Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-PE carriage status and presence of specific resistance genes. Dichotomous variables are expressed in frequencies and percentages, while continuous variables are in means with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in multivariate analyses to identify statistically significant factors that influence and determine KAP and ESBL-PE carriage. Covariates that were found to be statistically significant in the univariate analyses were included in the multivariate models. All tests of significance are performed at α=5%. Statistical analysis was carried out using R Version 1.1.383.[21]

Patient and Public Involvement

A group of 75 community dwellers partnered with us for the design and validation of the study questionnaire to ensure clarity and accuracy, production of informational material to support recruitment, and evaluation of the burden of the sample collection from the patient's perspective. Because there was no clear preference for the sample collection methodology, the study team decided to offer both options of rectal swab and stool collection to the study participants.

RESULTS

From June 2016 to April 2017, we randomly selected 2,200 households in Clementi for home visits. Out of these 2200 households, 693 (31.5%) agreed to participate, of whom 305 (44.0%) also provided stool samples or rectal swabs (Figure S1). Participant demographics are presented in Table 1. The median age of participants was 53 (IQR 38-66). A slight majority were women (393/693, 56.7%). The ethnic distribution of the participants was similar to the wider Singapore population, with 513 (74.0%) Chinese, 78 (11.3%) Malay, and 83 (12.0%) Indian. The majority had received at least secondary school education (534/693, 77.0%), and stayed in public housing apartments (661/693, 95.4%). The median number of occupants per household was 3 (IQR 2-4) persons. A quarter (25.3%, 175/693) reported having taken antibiotics in the past 6 months, and 102 (14.7%) had recently been hospitalised in the past 1 year.

Table 1. Demographics, medical background and antibiotic use of study participants

Characteristic		N (%) Total N=693
Age (median, IQR*)		53.0 (38.0-66.0)
Females		393 (56.7)
Race	Chinese	513 (74.0)
	Malay	78 (11.3)
	Indian	83 (12.0)
	Other ethnicities	19 (2.7)
Education level	Graduate	88 (12.7)
	Diploma	251 (36.2)
	Secondary	195 (28.1)
	Primary	122 (17.6)
	No Formal Education	37 (5.3)
Housing type	1-, 2 or 3-room public housing	334 (48.2)
	4 or 5- room public housing	327 (47.2)
	Private landed property	32 (4.6)
Number of occupants in the household	Overall (median, IQR)	3 (2-4)
	≤ 3 persons	369 (53.2)
	4-5 persons	257 (37.1)
	≥ 6 persons	67 (9.7)

Comorbidities	Any chronic illnesses	239 (34.5)
	Hypertension	105 (15.2)
	Hyperlipidemia	76 (11.0)
	Diabetes mellitus	67 (9.7)
Recent hospitalisation in the past 1 year		102 (14.7)
Antibiotic consumption	Within past 6 months	175 (25.3)
	More than 6 months ago	441 (63.6)
	Never taken antibiotics	77 (11.1)

*IQR- interquartile range, ^Immunocompromised – Use of chemotherapy, corticosteroids or immunosuppressants in the past 6 months

The survey revealed widespread misinformation about antibiotics, with a mean knowledge score of only 4.6 (IQR 3.0-6.0) out of 10 (Table S2). Although the majority of participants knew that viruses are the most common cause of upper respiratory tract infections, a significant proportion (335/693, 48.3%) believed that antibiotics could be used for viral infections and 385 (385/693, 55.6%) thought that the most common cause of diarrhoea was bacteria. The questionnaire also explored participants' compliance to the widely accepted view of completing antibiotic courses. The majority (554/693, 79.9%) said they would complete the course of antibiotics prescribed, while 13.7% (95/693) would stop taking antibiotics when they start to feel better, and 6.3% (44/693) preferred to seek the doctor's opinion before stopping the course. Most participants (564/693, 81.4%) were aware that antibiotics are prescription-only drugs in Singapore, but were unable to correctly answer questions related to AMR, with 82.5% (572/693) not knowing what causes AMR, and 63.2% (438/693) believing AMR was not present in Singapore. Level of education ($p<0.001$) and staying in larger housing ($p=0.037$)—the usual proxies for socio-economic status in Singapore—were independent factors associated with higher total knowledge scores. However, higher knowledge scores were not strongly related to participants' trust in primary care physicians (OR 1.08, 95%CI 0.97-1.20) or the

expectation of an antibiotic prescription for common viral infections (OR 0.98, 95%CI 0.96-1.0).

A large majority of the community continued to place trust in their primary care doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted healthcare professionals as their primary source of medical information, over the Internet, media and family and friends. There were no significant associations between demographic factors and attitude scores in contrast to the differences seen in knowledge scores.

In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and vomiting), although about half of the participants (294/693, 42.4% for cough and runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the doctor for common complaints lasting less than 1 week, only 18.5% (average 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not prescribed during the initial visit, very few (average 39/693, 5.6%) reported they would insist on antibiotic prescription or seek a second opinion. The only independent factor associated with the expectation of an antibiotic prescription was younger age (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3% (23/693) admitted to having previously shared antibiotics with family members and 5.5% (38/693) to having taken leftover antibiotics from a previous illness.

Asymptomatic carriage of ESBL-PE

Three hundred and five participants (305/693, 44.0%) provided rectal swabs or stool samples for microbiology cultures. The participants who provided stool samples were not significantly different from those who did not, in terms of age, gender and education level. Eighty participants (80/305, 26.2%, 95%CI: 21.5-31.6%) were found to carry at least one ceftriaxone non-susceptible *Enterobacteriaceae* isolate. One hundred and fifteen isolates were detected on the ESBL screening media, of which 93 were ceftriaxone resistant or intermediate *Enterobacteriaceae*. Six bacterial isolates were detected on the CRE screening media, none of which were confirmed to be carbapenemase-producing *Enterobacteriaceae*. The factors associated with ESBL-PE carriage from multivariate logistic regression analysis were residency overseas for more than 1 year (OR 3.3, 95%CI 1.6-6.9), with the most common location being other parts of Asia, scoring higher than 6 on the knowledge component in the questionnaire (OR 2.0 95%CI 1.03- 3.9) and having no left over antibiotics (OR 2.4, 95%CI 1.24-4.9). Interestingly, recent hospitalisation and reported antibiotic intake were not associated with ESBL-PE carriage (Table 2).

Table 2. Univariate analysis of demographic characteristics associated with carriage of ceftriaxone- resistant *Enterobactriaceae*

Factors		Total N=305	Carriers N=80	Non-carriers N=225	p-values
Age (median, IQR*)		54.0 (41.0-65.0)	56.0 (38.8-66.0)	54.0 (41.0-65.0)	0.79
Females (%)		169 (55.4)	46 (57.5)	123 (54.7)	0.76
Ethnicity (%)	Chinese	237 (77.7)	67 (83.8)	170 (75.6)	0.24
	Malay	28 (9.2)	3 (3.8)	25 (11.1)	
	Indian	30 (9.8)	7 (8.8)	23 (10.2)	
	Others	10 (3.3)	3 (3.8)	7 (3.1)	
Education (%)	No formal	11 (3.6)	4 (5.0)	7 (3.1)	0.45

	education				
	Primary	57 (18.7)	12 (15.0)	45 (20.0)	
	Secondary	93 (30.5)	21 (26.2)	72 (32.0)	
	Tertiary	110 (36.1)	31 (38.8)	79 (35.1)	
	Graduate	34 (11.1)	12 (15.0)	22 (9.8)	
Housing (%)	HDB 1- and 2-room	23 (7.5)	5 (6.2)	18 (8.0)	0.75
	HDB 3-room	115 (37.7)	32 (40.0)	83 (36.9)	
	HDB 4-room	98 (32.1)	24 (30.0)	74 (32.9)	
	HDB 5-room and Executive Apartment	47 (15.4)	11 (13.8)	36 (16.0)	
	Landed Property	22 (7.2)	8 (10.0)	14 (6.2)	
Pets (%)		33 (10.8)	7 (8.8)	26 (11.6)	0.75
Number of occupants in the household (mean, sd)		3.6 (1.6)	3.6 (1.6)	3.6 (1.6)	0.71
Stayed overseas for >1 year (%)		57 (18.7)	26 (32.5)	31 (13.8)	<0.001
Stayed in South, East or Southeast Asia for >1 year (%)		40 (13.1)	18 (22.5)	22 (9.8)	0.01
Travelled in the past >1 year (%)		178 (58.4)	47 (58.8)	131 (58.2)	1.0
Travelled in South, East or Southeast Asia in the past 1 year (%)		163 (53.4)	43 (53.8)	120 (53.3)	1.0
Any chronic illnesses (%)		127 (41.6)	33 (41.2)	94 (41.8)	1.0
Hospitalisation in the past 1 year (%)		43 (14.1)	14 (17.5)	29 (12.9)	0.41
Previous antibiotics intake (%)		282 (92.5)	76 (95.0)	206 (91.6)	0.45
Antibiotics in the last 6 months (%)		85 (27.9)	23 (28.8)	62 (27.6)	0.61
Knowledge score >6 (%)		89 (29.2)	33 (41.3)	56 (24.9)	0.01
No left over antibiotics (%)		211 (69.2)	63 (78.8)	148 (65.8)	0.04

*IQR- interquartile range

Out of the 93 ceftriaxone-resistant isolates, 17 were cefoxitin resistant, suggestive of AmpC β-Lactamase production. Only one *Enterobacter cloacae* complex isolate was resistant to ertapenem and was of intermediate susceptibility to meropenem (Table 3). This *Enterobacter cloacae* complex isolate was not a carbapenemase-producer based

on phenotypic and genotypic tests. Eighty-three (83/93, 89.2%) of these ESBL-PE isolates were *E. coli*. The majority of ESBL-PE remained susceptible to aminoglycosides including gentamicin (80/93, 86.0%) and amikacin (91/93, 97.8%) as well as nitrofurantoin (76/93, 81.7%), while ciprofloxacin (53/93, 57.0%) and Sulfamethoxazole-trimethoprim (32/93, 34.4%) resistance were more common.

Table 3. Antibiotic susceptibility of the ceftriaxone-resistant isolates

	E coli (N=83) N (%)	*Klebsiella* (N=6) N (%)	Others^ (N=4) N (%)	Total (N=93) N (%)
Piperacillin-tazobactam	73 (88.0)	4 (66.7)	1 (25.0)	78 (83.9)
Cefepime	35 (42.4)	3 (50)	2 (50.0)	40 (43.0)
Aztreonam	39 (47.0)	2 (33.3)	1 (25.0)	42 (45.2)
Amikacin	82 (98.8)	5 (83.3)	4 (100)	91 (97.8)
Gentamicin	75 (90.4)	3 (50)	2 (50.0)	80 (86.0)
Nitrofuratoin	73 (88.0)	2 (33.3)	1 (25.0)	76 (81.7)
Sulfamethoxazole-trimethoprim	32 (38.6)	0 (0)	0 (0)	32 (34.4)
Ciprofloxacin	48 (57.8)	4 (66.7)	1 (25.0)	53 (57.0)
Fosfomycin	63 (75.9)	1 (16.7)	0 (0)	64 (68.8)
Ertapenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)
Meropenem	83 (100)	6 (100)	3 (75.0)	92 (98.9)

^ Others include *Enterobacter* spp (2), *Proteus mirabillis* (1), *Raoultella ornithinolyitca* (1)

Molecular classification of ESBL-PE

Eighty (80/93, 85%) ESBL-PE isolates from unique participants underwent whole genome sequencing. When two or more isolates grew from a single subject's sample, *E. coli*, the commonest species observed, was selected to facilitate comparisons. Genotypic species determination from the sequence reads correlated completely with the results by MALDI-TOF MS or the Vitek-2 system. Seventy-one (71/80, 88.8%)

isolates were *E. coli*, of which the most common molecular type was sequence type (ST) 131 (11/71, 15.5%) (Table S5). The most frequently observed ESBL gene was CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27 (16/71, 22.5%). *E coli* ST131 were more resistant to fluoroquinolones than non-ST131 isolates (p=0.041). The only significant factor from the questionnaire associated with ESBL-producing *E. coli* ST131 carriage was having more children in the household, but the difference was marginal (mean 0.3±0.7 versus 0.8 ± 1.1, p=0.034).

DISCUSSION

We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy community dwellers in Singapore, twice the rate found in an earlier study in 2014 of patients at an emergency department.[22] Similar rises have been observed globally.[3] Although these figures are lower than the reported prevalence of over 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly regulated antibiotic prescription system similar to Europe and the US where only registered medical practitioners are allowed to prescribe antibiotics, and they must be purchased from licensed dispensers. We did not find any association between fecal carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a city-state and overseas travel is very common, making it hard to detect such a relationship when frequent trips to neighbouring countries are made. However, past residency overseas was strongly associated with colonisation, especially those who lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). Distinctions should be made in future studies on overseas travels and AMR carriage in terms of

duration and frequency of travel, in addition to destinations. The possibility of substantial acquisition of MDRO colonisation and infection through overseas exposure[24,25] once again highlights the urgent need for a regional, collaborative approach to tackling the problem of AMR.

In addition, we did not find an association between recent antibiotic intake in the past 6 months and ESBL-PE carriage. This is inconsistent with previous reports showing that consumption of certain classes of antibiotics such as beta-lactams and fluoroquinolones are risk factors for predispositions to ESBL-PE carriage. [26,27] The possible reasons could be due to the relatively small number of participants who had recent antibiotic intake (85/305, 27.9%), so we were not able to distinguish the specific classes of antibiotics taken by the participants. It is also possible that the dominance of a hyperendemic community-associated clone rather than antibiotic selection pressure alone contributed to this finding.

Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL mechanism, echoing the global dissemination of this hyperendemic clone, especially in the community.[28] Similar reports showed 11.1% (32/287) in China[29] and 4.1% (8/193) in Thailand[30] have been published. The reason for the rapid worldwide expansion and long-term persistence of *E. coli* ST131 is thought to be due to compensatory mutations within the core genome counterbalancing the fitness cost associated with IncF plasmids, thus sustaining its spread even in the absence of direct antibiotic selection pressure.[31] These *E. coli* ST131 are not just prevalent colonisers but have also associated with invasive bloodstream infections in hospitalized patients

in Australia, New Zealand and Singapore.[32] It will be important to better understand the evolutionary ecology and transmission dynamics of this emerging clone.

This study also revealed widespread misconceptions about the utility of antibiotics for viral infections, consistent with the findings of a global survey conducted by the WHO in 2015.[33] We also found that, the public continues to place trust in their primary care doctors and their recommendations. This dependence on physicians is in contrast to doctors' perceptions of patient expectations for antibiotic prescriptions.[34] This discordance has been previously described and is thought to be due to the lack of empowerment of the patient and the erroneous attribution of patient satisfaction to antibiotic prescription rather than a focus on better patient-doctor communication.[35,36]

Engaging and educating both the prescribers and the public may reduce inappropriate antibiotic use,[37,38] and has been identified as a key strategy by the WHO and the UK to tackle AMR.[39,40] One of the most striking findings of this study is that having both the knowledge that antibiotic courses should be completed and not having left over antibiotics is independently associated with the carriage of ESBL-PE. Though these relationships cannot be viewed as causal given the complexities in the emergence and transmission of AMR, there is emerging evidence supporting short course antibiotic therapies, even for severe infections such as bacteremia, given the collateral damage that antibiotics have on host microbiome.[41] The current WHO recommendation remains that full courses of antibiotics should be completed to prevent the onset of resistance.[33] Similar messages are advocated in national

campaigns launched in Australia,[42] the United States[43] and Europe.[44] Given that the minimum effective treatment durations have not been determined for many infections and that a significant proportion of antibiotic prescriptions are inappropriate, the emphasis on completing the course of antibiotics to prevent resistance may have to be re-examined.

To our knowledge, this is the first study that explored antibiotic consumption behavior with the acquisition of MDRO at a community level. This novel approach has the potential to guide clinicians and policy makers in identifying directly actionable interventions for the population. The main weakness of our study is that the questionnaire data is self-reported and subjected to recall and interviewer biases. We minimised these errors by designing specific questions that are carefully constructed to maximize accuracy and completeness, and all interviewers were trained to adhere to the question and answer format strictly. Further research using antibiotic prescription databases can potentially overcome some of the intrinsic biases arising from cross-sectional questionnaires.

CONCLUSION

There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore, especially with *E. coli* ST131 carrying CTX-M. This is correlated with KAP of antibiotic use, especially with the practice of finishing full courses of antibiotics, and prolonged residency in other parts of Asia. Innovative approaches to control AMR that take into account transboundary transmission of resistance and clinical trials to determine the appropriate duration of antimicrobial therapy will be critical to control

the emergence of these resistant clones which have contributed significantly to the current global antibiotic resistance crisis.

CONTRIBUTOR AND GUARANTOR INFORMATION

YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK performed microbiological testing. RTHO planned and conducted genomic sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW performed data analysis. All participated in the writing of the script, and affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained. YM and IS accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and no others meeting the criteria have been omitted.

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COMPETING INTERESTS DECLARATION

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

DATA SHARING

The authors commit to making the relevant anonymised patient level data available on reasonable request.

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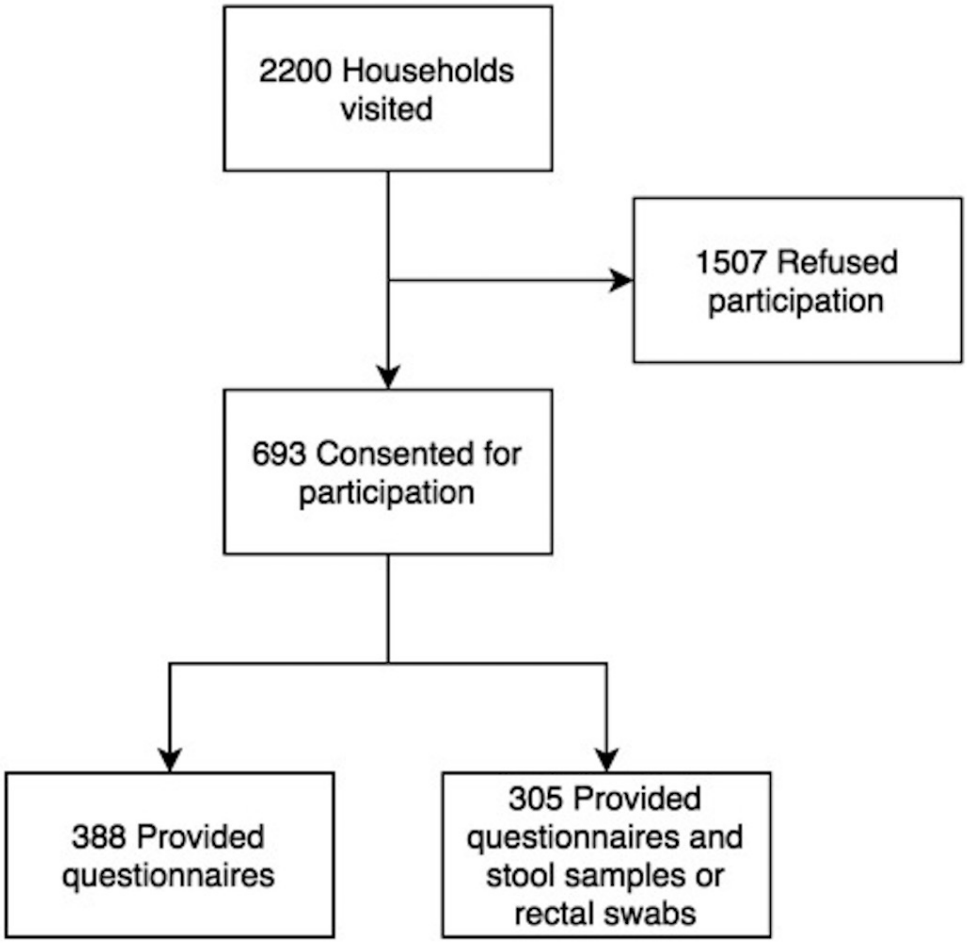

Figure S1. Participant recruitment flowchart

90x87mm (300 x 300 DPI)

Table S1. Study questionnaire

Section 1: Background Data

1. Demographic Data
1.1 Age
1.2 Gender - Male or Female
2. Race - Chinese or Malay or Indian or Others
3. Educational Background
2.1 Highest Education Level Attained- Primary Education or Secondary Education or Tertiary Education or Graduate Education or No formal education
4. Have you ever studied a healthcare-related course? (Medicine, Traditional Chinese Medicine, Therapy, Nursing) - Yes or No
3 Occupation and Financial Status
5. Ocupation:
4 Accommodation
4.1 Housing type- Public housing (1-Room or 2-Room or 3-Room or 4-Room or 5-Room or Executive Apartment) or Landed property
4.2 How many occupants are there living in your house? (including you) Number of Occupants:
4.3.1 How many people in the household are in the following age group? Less than 12 years old:
4.3.2 How many people in the household are in the following age group? More than 65 years old:
6. Do you currently have any dogs or cats at home? - Yes or No
5 Travel history

5.3 Have you travelled to the following places within the past 6 months? – Yes or No

5.3.1 If yes, which of the following places have you been to? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

5.4 Have you lived anywhere else for more than 1 year? – Yes or No

6 If yes, did you live in the following areas? (You may select more than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or North America and/ or Middle East or Others:

7 Medical History

6.1 Do you have any of the following? (You can choose more than one of the following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids, Immunosuppressants etc) and/ or Other medical conditions or None of the above

6.2 When was your last hospitalisation? - Never been hospitalised before or Hospitalised before

6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

6.2.2 How long was your stay? Duration:

6.3 Have you used antibiotics before? - Have never used antibiotics before or Used antibiotics before

6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6

months or More than 6 months ago

Section 2: Assessment of Antibiotic Practices

1. Assessing Health-Seeking and Antibiotic-Seeking Behaviours

Scenario 1: Cough and Runny Nose

1.1.1 Would you go to the doctor for a cough and runny nose that lasted less than 1 week? – Yes or No or I am not sure

1.1.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.1.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

Scenario 2: Diarrhoea and Vomiting

1.2.1 Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than a week? – Yes or No or I am not sure

1.2.2 In the above scenario, did you expect the doctor to prescribe antibiotics to help with the recovery? – Yes or No or I am not sure

1.2.3 If the doctor you were seeing does not prescribe you antibiotics for the symptoms above, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription? – Yes or No or I am not sure

2. Assessing Practices of Disposal and Storage of Antibiotics

2.1 What do you usually do with leftover antibiotics? - Usually do not have leftovers

or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the rubbish bin or Others:

3. Assessing Alternative Antibiotic Practices

3.1 Have you ever shared antibiotics with someone else? – Yes or No

3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes or No

Section 3: Attitude Assessment

1. Attitudes Towards Healthcare Provider Prescription

1.1 Sometimes my doctor prioritises what is beneficial for him over my medical needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.3 My doctor is always honest when telling me about all the available treatments for my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree

2. Attitudes Towards Potential Educational Interventions

2.1 Which of the following sources of medical information do you trust most? - Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists) or Family and Friends or Online Medical Sources or Television Programmes and Advertisements or Radio Programmes and Advertisements

Section 4: Knowledge Assessment

1. Knowledge on Function of Antibiotics

1.1 Antibiotics are medicines that can treat viral infections. – True or False or I am not sure

1.2 Antibiotics are medicines that can treat bacterial infections. – True or False or I am not sure

1.3 Antibiotics are medicines that can treat fungal infections. – True or False or I am not sure

2. Knowledge on Agents of Infection

2.1 Which of the following most commonly causes running nose and cough? – Virues or Bacteria or I am not sure

2.2 Which of the following most commonly causes diarrhoea? – Virues or Bacteria or I am not sure

3. Knowledge on Proper Use of Antibiotics

3.1 Antibiotics can be obtained at the pharmacist without any prescription. - True or False or I am not sure

3.2 Antibiotics can be stopped when: - You start to feel better or You finish the entire course or You head back to the doctor and he tells you that you can stop

4. Knowledge on Concept of Antibiotic Resistance

4.1 Do you understand what is antibiotic resistance? – Yes or No or I am unsure

4.1.1 If yes, describe what causes antibiotic resistance?

4.2 Which of the following is a consequence of antibiotic resistance? (choose only ONE option) - Antibiotics become more effective at treating infections or Antibiotics become less effective at treating infections or Your body immunity

becomes weaker or Your body immunity becomes stronger

4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am unsure

Table S2. Assessment of knowledge

Questions			N (%) Total N= 693
2.1.1	Antibiotics are medicines that can treat viral infections.	False	149 (21.5%)
		True	335 (48.3%)
		Unsure	209 (30.2%)
2.1.2	Antibiotics are medicines that can treat bacterial infections.	True	419 (60.5%)
		False	50 (7.2%)
		Unsure	224 (32.3%)
2.1.3	Antibiotics are medicines that can treat fungal infections.	False	157 (22.7%)
		True	194 (28.0%)
		Unsure	342 (49.4%)
2.1.4	Which of the following most commonly causes running nose and cough.	Viruses	352 (50.8%)
		Bacteria	130 (18.8%)
		Unsure	211 (30.4%)
2.1.5	Which of the following most commonly causes diarrhoea?	Viruses	98 (14.1%)
		Bacteria	385 (55.6%)
		Unsure	210 (30.3%)
2.1.6	Antibiotics can be stopped when…	You finish the entire course	554 (79.9%)
		When you feel better	95 (13.7%)
		Consult the doctor	44 (6.3%)
2.1.7	Antibiotics can be obtained at the pharmacist without any prescription.	False	564 (81.4%)
		True	29 (4.2%)
		Unsure	100 (14.4%)

2.1.8	What causes antimicrobial resistance? (Open ended)	Inappropriate use of antibiotics	121 (17.5%)
		Wrong or unsure	572 (82.5%)
2.1.9	Which of the following is a consequence of antibiotic resistance?	Antibiotics becoming more effective at treating infections	280 (40.4%)
		Antibiotics becoming less effective at treating infections	111 (16.0%)
		Your body immunity becomes weaker	235 (33.9%)
		Your body immunity becomes stronger	67 (9.7%)
2.1.10	Antibiotic resistance is not present in Singapore yet.	False	255 (36.8%)
		True	77 (11.1%)
		Unsure	361 (52.1%)

Table S3. Assessment of attitude toward primary care

Questions			N (%) N= 693
2.2.1	Sometimes my doctor prioritises what is beneficial for him over my medical needs	Strongly agree	14 (2.0)
		Agree	109 (15.7)
		Neutral	145 (20.9)
		Disagree	335 (48.3)
		Strongly disagree	90 (13.0)
2.2.2	My doctor's medical skills are not as good as they should be	Strongly agree	10 (1.4)
		Agree	83 (12.0)
		Neutral	150 (21.6)
		Disagree	373 (53.8)
		Strongly disagree	77 (11.1)
2.2.3	My doctor is always honest when telling me about all the available treatments for my condition	Strongly agree	100 (14.4)
		Agree	427 (61.6)
		Neutral	115 (16.6)
		Disagree	45 (6.5)
		Strongly disagree	6 (0.9)
2.2.4	I have no worries about putting my life in my doctor's hands	Strongly agree	110 (15.9)
		Agree	363 (52.4)
		Neutral	135 (19.5)
		Disagree	74 (10.7)
		Strongly disagree	11 (1.6)
2.2.5	Which of the following sources of medical information do you trust most?	Healthcare professional's advice	627 (90.6)
		Family and friends	36 (5.2)
		Online medical sources	24 (3.5)

		Television programmes and advertisements	4 (0.6)
		Radio programmes and advertisements	1 (0.1)

Table S4. Assessment of practices

Questions			N (%) Total N= 693
2.3.1.1	Would you go to the doctor for a cough and runny nose that lasted less than 1 week	Yes	294 (42.4)
		No	377 (54.4)
		Unsure	22 (3.2)
2.3.1.2	Would you go to the doctor for diarrhoea, vomiting and stomach pain that lasted less than 1 week?	Yes	414 (59.7)
		No	262 (37.8)
		Unsure	17 (2.5)
2.3.2.1	Would you expect the doctor to prescribe antibiotics for cough and runny nose that lasted less than 1 week to help with the recovery?	Yes	136 (19.6)
		No	508 (73.3)
		Unsure	49 (7.1)
2.3.2.2	Would you expect the doctor to prescribe antibiotics for diarrhoea, vomiting and stomach pain that lasted less than 1 week to help with the recovery?	Yes	120 (17.3)
		No	501 (72.3)
		Unsure	72 (10.4)
2.3.3.1	If the doctor you were seeing does not prescribe you antibiotics for cough and runny nose that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	37 (5.3)
		No	619 (89.3)
		Unsure	37 (5.3)
2.3.3.2	If the doctor you were seeing does not prescribe you antibiotics for diarrhea vomiting and stomach pain that lasted less than 1 week, would you seek another doctor's opinion or firmly request the doctor for an antibiotic prescription?	Yes	40 (5.8)
		No	615 (88.7)
		Unsure	38 (5.5)
2.3.4.1	What do you usually do with left over antibiotics?	No left overs	476 (68.7)
		Disposal in rubbish bin	130 (18.8)
		Keep for future use	60 (8.7)

		Unsure	19 (2.7)
		Pour down sink or toilet bowl	8 (1.2)
2.3.4.2	Have you ever shared antibiotics with anyone else?	Yes	23 (3.3)
		No	670 (94.5)
2.3.4.3	Have you ever taken leftover antibiotics from a previous course of illness?	Yes	38 (5.5)
		No	655 (9.5)

Table S5. Molecular classification of ceftriaxone-resistant E coli isolates

		E coli N=71 (%)		p-value
		ST131 N=11 (%)	Non ST131 N=60 (%)	
Number of resistant genes (mean ± sd)		1.2 ± 0.4	1.9 ± 0.8	0.0012
ESBL genes				
CTXM	15	4 (36.4)	17 (28.3)	0.72
	27	7 (63.6)	9 (15.0)	
	14	0 (0.0)	10 (16.7)	
	55	0 (0.0)	9 (15.0)	
	8	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	9 (15.0)	
	None	0 (0.0)	3 (5.0)	
SHV	12	0 (0.0)	3 (5.0)	1.0
	None	11 (100.0)	57 (95.0)	
TEM	206	1 (9.1)	11 (18.3)	0.11
	198	0 (0.0)	3 (5.0)	
	Others	0 (0.0)	15 (25.0)	

	None	10 (90.9)	31 (51.7)	
OXA		1 (9.1)	3 (5.0)	1.0
Quinolone resistance		8 (72.7)	21 (35.0)	0.041

* Non-ST131 sequence types are: 38 (N=8), 1193 (N=5), 10 (N=4), 48 (N=3), other (N=35), none (N=5)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-8
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7-8
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	4-5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Supplementary material
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	9-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-15
		(*b*) Report category boundaries when continuous variables were categorized	9-15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19-20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.