

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023859
Article Type:	Research
Date Submitted by the Author:	02-May-2018
Complete List of Authors:	<p>Mo, Yin; National University Health System, Division of Infectious Diseases, University Medicine Cluster</p> <p>Seah, Ivan; National University Singapore Yong Loo Lin School of Medicine</p> <p>Lye, Pei Shi Priscillia ; National University Singapore Yong Loo Lin School of Medicine</p> <p>Kee, Xiang Lee Jamie ; National University Singapore Yong Loo Lin School of Medicine</p> <p>Wong, Kien Yee Michael; National University Singapore Yong Loo Lin School of Medicine</p> <p>Ko, Kwan Ki Karrie ; Singapore General Hospital, Department of Microbiology</p> <p>Ong, Rick Twee-Hee ; National University Singapore Saw Swee Hock School of Public Health</p> <p>Tambyah, Paul; National University Health System, Division of Infectious Diseases, University Medicine Cluster; National University of Singapore, Department of Medicine</p> <p>Cook, Alex R ; National University Singapore Saw Swee Hock School of Public Health</p>
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Antimicrobial resistance, Duration of antibiotic treatment

SCHOLARONE™
Manuscripts

1
2
3 Finishing the prescribed course of antibiotics is associated with Extended Spectrum
4 Beta-Lactamase producing *Enterobacteriaceae* carriage – results of a Singapore
5 Community Survey
6
7
8
9
10
11
12

13 Yin MO^{1*}, Ivan SEAH^{2*}, Pei Shi Priscillia LYE², Xiang Lee Jamie KEE², Kien Yee
14 Michael WONG², Kwan Ki Karrie KO³, Rick Twee-Hee ONG⁴, Paul Anantharajah
15 TAMBYAH^{1,5}, Alex R COOK⁴
16
17
18
19
20
21
22
23

24 1. Division of Infectious Disease, University Medicine Cluster, National University
25 Hospital, 5 Lower Kent Ridge Road Singapore 119074; 2. Yong Loo Lin School of
26 Medicine, 1E Kent Ridge Road Singapore 119228; 3. Department of Microbiology,
27 Singapore General Hospital, Outram Road Singapore 169608; 4. Saw Swee Hock
28 School of Public Health, National University of Singapore, 21 Lower Kent Ridge
29 Road Singapore 119077; 5. Department of Medicine, National University of
30 Singapore, 21 Lower Kent Ridge Road Singapore 119077
31
32
33
34
35
36
37
38
39
40

41 * Authors contributed to this work equally.
42
43
44
45

46 Correspondence to: Yin Mo yin_mo@nuhs.edu.sg +65 67795555
47
48
49

50 Word count: 2736
51
52
53
54
55
56
57
58
59
60

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,

1
2
3 we randomly selected 2,200 households in Clementi for home visits. The study team
4
5 returned to non-responding households for up to three times on separate days to
6
7 maximise the response rate. One representative adult above 21 years old in each
8
9 household was invited to participate in this cross-sectional study; all consenting
10
11 individuals undertook a questionnaire, while some additionally consented to provide a
12
13 rectal swab or stool sample. Ethical approval was obtained from National University
14
15 of Singapore Institutional Review Board (Reference number B-16-245).
16
17
18
19

20 **Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake** 21 **and health-seeking behaviour**

22
23
24 We conducted a questionnaire study to assess the KAP of participants towards
25
26 antibiotic use. A 40-item questionnaire was developed after performing a thorough
27
28 literature review of comparable studies.[9–14] This was then validated by a pilot
29
30 study involving 75 community-dwelling volunteers to ensure fluency and accuracy in
31
32 question design and language. A team of thirty-three investigators was trained to
33
34 administer the survey face-to-face.
35
36
37
38
39

40 The questionnaire comprised four main sections. The first covered socio-demographic
41
42 data and recent antibiotic intake. The second was an assessment of antibiotic
43
44 consumption practices, in which two hypothetical scenarios of diarrhoea and upper
45
46 respiratory tract symptoms were presented, and participants were asked if they would
47
48 visit the doctor should they experience these symptoms for less than 1 week, if they
49
50 would expect or insist on an antibiotic prescription from the doctor's visit, and if they
51
52 would seek a second opinion if antibiotics were not prescribed. The third component
53
54 assessed participants' attitudes and trust towards primary care healthcare providers,
55
56
57
58
59

1
2
3 and was adapted from a validated questionnaire from Hall *et al.*[15] The last
4
5 component examined participants' knowledge on AMR. The full questionnaire and
6
7 grading system can be found in Table S1.
8
9

10 11 **Bacterial isolation and antibiotic susceptibility testing**

12
13 The study team requested fresh stool samples or rectal swabs from all study
14
15 participants. The samples of those who consented were collected from the participants
16
17 within 24 hours of production and stored centrally at 0-4°C prior to microbiological
18
19 processing. All sample processing was carried out in the Singapore General Hospital
20
21 Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagar*TM
22
23 *ESBL* and *CHROMID*[®] *CARBA SMART* (*bioMerieux*) media to detect cephalosporin-
24
25 resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24
26
27 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and
28
29 used for subsequent species identification and antibiotic susceptibility testing. Species
30
31 identification was done by matrix-assisted laser desorption/ionization-time of flight
32
33 mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 (*bioMerieux*) system.
34
35
36
37
38

39
40 Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, cefoxitin, cefepime,
41
42 amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin,
43
44 nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin,
45
46 ertapenem and meropenem were assessed by the disc diffusion method and
47
48 interpreted according to the Clinical Laboratory Standards Institute (CLSI)
49
50 criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth
51
52 generation cephalosporins were identified as potential ESBL producers, while those
53
54 not susceptible to any carbapenem were identified as potential carbapenemase
55
56
57
58
59
60

1
2
3 producers. Potential carbapenemase producers were tested phenotypically for
4 carbapenemase production by modified Hodge test and KPC/MBL and OXA-48
5 Confirm Kit (ROSCO). All potential carbapenemase producers were also subjected to
6 the Xpert[®] Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM
7 carbapenemase gene sequences.
8
9
10
11
12

13 14 15 16 **Whole genome sequencing of ESBL-producing *Enterobacteriaceae***

17 DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially
18 ESBL- or carbapenemase- producers, with sequencing libraries for each isolate
19 prepared as per manufacturer's recommendation to be multiplexed sequenced on the
20 Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs,
21 having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads
22 was performed using the SPAdes Genome Assembler.[17] Bacterial species were
23 identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence
24 types (MLSTs) were determined by a customized script utilising BLAST search for
25 identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial
26 resistance owing to the existence of specific gene sequences were performed using
27 SRST2.[20]
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **Statistical Analysis**

46 Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-
47 PE carriage status and presence of specific resistance genes. Dichotomous variables
48 are expressed in frequencies and percentages, while continuous variables are in means
49 with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's
50 exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric
51
52
53
54
55
56
57
58
59
60

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 $\alpha=5\%$. 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

1
2
3 better, and 6.3% (44/693) preferred to seek the doctor's opinion before stopping the
4
5 course. Most participants (564/693, 81.4%) were aware that antibiotics are
6
7 prescription-only drugs in Singapore, but were unable to correctly answer questions
8
9 related to AMR, with 82.5% (572/693) not knowing what causes AMR, and 63.2%
10
11 (438/693) believing AMR was not present in Singapore. The level of education
12
13 (p<0.001) and staying in larger housing (p=0.037)—the usual proxy for socio-
14
15 economic status in Singapore—were independent factors associated with higher total
16
17 knowledge scores. However, higher knowledge scores were not strongly related to
18
19 participants' trust in primary care physicians (OR 1.08, 95%CI 0.97-1.20) or the
20
21 expectation of an antibiotic prescription for common viral infections (OR 0.98,
22
23 95%CI 0.96-1.0).
24
25
26
27
28

29 A large majority of the community continued to place trust in their primary care
30
31 doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted
32
33 healthcare professionals as their primary source of medical information, over the
34
35 Internet, media and family and friends. There were no significant associations
36
37 between demographic factors and attitude scores in contrast to the differences seen in
38
39 knowledge scores.
40
41
42
43

44 In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and
45
46 vomiting), although about half of the participants (294/693, 42.4% for cough and
47
48 runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the
49
50 doctor for common complaints lasting less than 1 week, only 18.5% (average
51
52 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not
53
54 prescribed during the initial visit, very few (average 39/693, 5.6%) reported they
55
56
57
58
59
60

1
2
3 would insist on antibiotic prescription or seek a second opinion. The only independent
4 factor associated with the expectation of an antibiotic prescription was younger age
5 (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover
6 antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers
7 antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing
8 with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3%
9 (23/693) admitted to having previously shared antibiotics with family members and
10 5.5% (38/693) to having taken leftover antibiotics from a previous illness.
11
12
13
14
15
16
17
18
19
20
21

22 **Asymptomatic carriage of ESBL-PE**

23
24 Three hundred and five participants (305/693, 44.0%) provided rectal swabs or stool
25 samples for microbiology cultures. Eighty participants (80/693, 26.2%, 95%CI: 21.5-
26 31.6%) were found to carry at least one ceftriaxone non-susceptible
27 *Enterobacteriaceae* isolate. One hundred and fifteen isolates were detected on the
28 ESBL screening media, of which 93 were ceftriaxone resistant or intermediate
29 *Enterobacteriaceae*. Six bacterial isolates were detected on the CRE screening media,
30 none of which were confirmed to be carbapenemase-producing *Enterobacteriaceae*.
31 The factors associated with ESBL-PE carriage from multivariate logistic regression
32 analysis were residency overseas for more than 1 year (OR 3.3, 95%CI 1.6-6.9), with
33 the most common location being other parts of Asia, scoring higher than 6 on the
34 knowledge component in the questionnaire (OR 2.0 95%CI 1.03- 3.9) and having no
35 left over antibiotics (OR 2.4, 95%CI 1.24-4.9). Interestingly, recent hospitalisation
36 and reported antibiotic intake were not associated with ESBL-PE carriage (Table 2).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

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 ceftioxin 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

	<i>E coli</i> (N=83) N (%)	<i>Klebsiella</i> (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)
Nitrofurantoin	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)
Fosfomicin	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 mirabilis* (1), *Raoultella*

ornithinolytica (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

	<i>E coli</i> 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)	0.72
	27	7 (63.6)	
	14	0 (0.0)	
	55	0 (0.0)	
	8	0 (0.0)	
	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

1
2
3 exposure[24,25] once again highlights the urgent need for a regional, collaborative
4
5 approach to tackling the problem of AMR.
6
7

8
9 Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131
10
11 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL
12
13 mechanism, echoing the global dissemination of this hyperendemic clone, especially
14
15 in the community.[26] Similar to reports from communities was 11.1% (32/287) in
16
17 China[27] and 4.1% (8/193) in Thailand[28] have been published. The reason for the
18
19 rapid worldwide expansion and long-term persistence of *E. coli* ST131 is thought to
20
21 be due to compensatory mutations within the core genome counterbalancing the
22
23 fitness cost associated with IncF plasmids, thus sustaining its spread even in the
24
25 absence of direct antibiotic selection pressure.[29] These *E. coli* ST131 are not just
26
27 prevalent colonisers but have also associated with invasive bloodstream infections in
28
29 hospitalized patients in Australia, New Zealand and Singapore.[30] It will be
30
31 important to better understand the evolutionary ecology and transmission dynamics of
32
33 this emerging clone.
34
35
36
37
38

39 This study also revealed widespread misconceptions about the utility of antibiotics for
40
41 viral infections, consistent with the findings of a global survey conducted by the
42
43 WHO in 2015.[31] We also found that, the public continues to place trust in their
44
45 primary care doctors and their recommendations. This dependence on physicians is in
46
47 contrast to doctors' perceptions of patient expectations for antibiotic
48
49 prescriptions.[32] This discordance has been previously described and is thought to be
50
51 due to the lack of empowerment of the patient and the erroneous attribution of patient
52
53
54
55
56
57
58
59
60

1
2
3 satisfaction to antibiotic prescription rather than a focus on better patient-doctor
4
5 communication.[33,34]
6
7

8
9 Engaging and educating both the prescribers and the public may reduce inappropriate
10 antibiotic use,[35,36] and has been identified as a key strategy by the WHO and the
11 UK to tackle AMR.[37,38] One of the most striking findings of this study is that
12
13 having a higher knowledge score and not having left over antibiotics were
14
15 independent risk factors for carriage of ESBL-PE. The current WHO recommendation
16
17 remains that full courses of antibiotics should be completed to prevent the onset of
18
19 resistance.[31] Similar messages are advocated in national campaigns launched in
20
21 Australia,[39] Canada,[40] the United States[41] and Europe.[42] However,
22
23 increasing evidence is emerging supporting shorter duration of antibiotics for
24
25 common infections.[43] The impact of prolonged antibiotic use on the host flora is
26
27 often underestimated.[43] Given that the minimum effective treatment durations have
28
29 not been determined for many infections and that a significant proportion of antibiotic
30
31 prescriptions are inappropriate, the whole question about “completing the course” of
32
33 antibiotics may have to be re-examined.
34
35
36
37
38
39
40
41

42 To our knowledge, this is the first study that explored antibiotic consumption
43
44 behavior with the acquisition of MDRO at a community level. This novel approach
45
46 has the potential to guide clinicians and policy makers in identifying directly
47
48 actionable interventions for the population. The main weakness of our study is that
49
50 the questionnaire data is self-reported and subjected to recall and interviewer biases.
51
52 We minimised these errors by designing specific questions that are carefully
53
54
55
56
57
58
59
60

1
2
3 constructed to maximize accuracy and completeness, and all interviewers were trained
4
5 to adhere to the question and answer format strictly.
6
7

8 9 **CONCLUSION**

10
11 There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore,
12
13 especially with *E. coli* ST131 carrying CTX-M. Innovative approaches to control
14
15 AMR that take into account transboundary transmission of resistance and clinical
16
17 trials to determine the appropriate duration of antimicrobial therapy will be critical to
18
19 control the emergence of these resistant clones which have contributed significantly to
20
21 the current global antibiotic resistance crisis.
22
23
24
25

26 27 **CONTRIBUTOR AND GUARANTOR INFORMATION**

28
29 YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the
30
31 study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK
32
33 performed microbiological testing. RTHO planned and conducted genomic
34
35 sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW
36
37 performed data analysis. All participated in the writing of the script, and affirm that
38
39 the manuscript is an honest, accurate, and transparent account of the study being
40
41 reported; that no important aspects of the study have been omitted; and that any
42
43 discrepancies from the study as originally planned have been explained. YM and IS
44
45 accept full responsibility for the work and/or the conduct of the study, had access to
46
47 the data, and controlled the decision to publish. The corresponding author attests that
48
49 all listed authors meet authorship criteria and no others meeting the criteria have been
50
51 omitted.
52
53
54
55
56
57
58
59
60

ACKNOWLEDGEMENT

The authors thank the study team members, Ang Chen Xiang, Anne Goei Hui Yi, Charmaine Loh Hui Yun, Cheong Shao Wei Dominic, Chew Shi Jie, Chong Yvette, Choo Hui Min Charlotte, Choo Xin Yi, Daveraj Sivasegaran, Dean Krishen Sethi, Joshua Tan Teck Chin, Keith Ching Wei Jie, Khoo Chun Yuet, Krystal Khoo Oon Hui, Lai Jieru, Liew Yi Song Terence, Lim Li Liang Joshua, Lok Si Ying Andrea, Lynette Sim Pei Shuen, Michelle Sim Yan Lin, Mok Charlene, Ong Yuxuan Daniel, Ong Zheng Xuan, Quek Keng Liang, R Krishnapriya, Sophia Ng Shuen Yii, Tan Fang Min Grace, Tan Jian Wei, Tan Pei Min Mabelleline, Tay Yiling Elaine, Tey Min Li, Wu Yanlin, Zhou Lingyue, for their contributions in carrying out home visits, interviews and sample collections.

COPYRIGHT/LICENCE FOR PUBLICATION

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, a worldwide licence to the Publishers and its licensees in the perpetuity, in all forms, formats and media (whether known now or created in the future), to i) publish, reproduce, distribute display and store the Contribution, ii) translate the Contribution into other languages, create adaptations, reprints, include within collections and create summaries, extracts and/or, abstracts of the Contribution, iii) create any other derivative work(s) based on the Contribution, iv) to exploit all subsidiary rights in the Contribution, v) the inclusion of electronic links from the Contribution to third party material where-ever it may be located; and, vi) licence any third party to do any or all of the above.

FUNDING

1
2
3 Data collection was supported from funding from the Infectious Diseases Research
4 Fund, National University of Singapore (NUS), and the Saw Swee Hock School of
5 Public Health (SSHSPH). RTHO received funding support from the SSHSPH, NUS.
6
7
8
9 ARC was supported by the Singapore Population Health Improvement Centre.
10

11 **COMPETING INTERESTS DECLARATION**

12
13 All authors have completed the ICMJE uniform disclosure form at
14 www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any
15
16 organisations that might have an interest in the submitted work in the previous three
17
18 years; no other relationships or activities that could appear to have influenced the
19
20 submitted work.
21
22
23
24
25

26 **DATA SHARING**

27
28 The authors commit to making the relevant anonymised patient level data available on
29
30 reasonable request.
31
32
33
34

35 **REFERENCES**

- 36
37 1 Tacconelli E, Carrara E, Savoldi A, *et al.* Discovery, research, and
38 development of new antibiotics: the WHO priority list of antibiotic-resistant
39 bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27.
40
41 doi:10.1016/S1473-3099(17)30753-3
42
43
44
45 2 Ruppé É, Woerther P-L, Barbier F. Mechanisms of antimicrobial resistance in
46 Gram-negative bacilli. *Ann Intensive Care* 2015;**5**:61. doi:10.1186/s13613-015-
47
48 0061-0
49
50
51
52 3 Karanika S, Karantanos T, Arvanitis M, *et al.* Fecal Colonization With
53 Extended-spectrum Beta-lactamase–Producing *Enterobacteriaceae* and Risk
54
55
56
57
58
59
60

- 1
2
3 Factors Among Healthy Individuals: A Systematic Review and Metaanalysis.
4
5 *Clin Infect Dis* 2016;**63**:310–8. doi:10.1093/cid/ciw283
6
- 7 4 Reddy P, Malczynski M, Obias A, *et al.* Screening for Extended-Spectrum -
8
9 Lactamase-Producing Enterobacteriaceae among High-Risk Patients and Rates
10
11 of Subsequent Bacteremia. *Clin Infect Dis* 2007;**45**:846–52.
12
13 doi:10.1086/521260
14
- 15 5 Troché G, Toly L-M, Guibert M, *et al.* Detection and Treatment of Antibiotic-
16
17 Resistant Bacterial Carriage in a Surgical Intensive Care Unit: A 6-Year
18
19 Prospective Survey. *Infect Control Hosp Epidemiol* 2005;**26**:161–5.
20
21 doi:10.1086/502521
22
23
- 24 6 Coker RJ, Hunter BM, Rudge JW, *et al.* Emerging infectious diseases in
25
26 southeast Asia: regional challenges to control. *Lancet* 2011;**377**:599–609.
27
28 doi:10.1016/S0140-6736(10)62004-1
29
30
- 31 7 Zellweger RM, Carrique-Mas J, Limmathurotsakul D, *et al.* A current
32
33 perspective on antimicrobial resistance in Southeast Asia. *J Antimicrob*
34
35 *Chemother* 2017;**72**:2963–72. doi:10.1093/jac/dkx260
36
37
- 38 8 Singapore Department of Statistics. Singapore Residents by Planning
39
40 Area/Subzone, 2015. 2015.
- 41 9 Pan DST, Huang JH, Lee MHM, *et al.* Knowledge, attitudes and practices
42
43 towards antibiotic use in upper respiratory tract infections among patients
44
45 seeking primary health care in Singapore. *BMC Fam Pract* 2016;**17**:148.
46
47 doi:10.1186/s12875-016-0547-3
48
49
- 50 10 Scaioli G, Gualano MR, Gili R, *et al.* Antibiotic Use: A Cross-Sectional
51
52 Survey Assessing the Knowledge, Attitudes and Practices amongst Students of
53
54 a School of Medicine in Italy. *PLoS One* 2015;**10**:e0122476.
55
56
57
58
59
60

- 1
2
3 doi:10.1371/journal.pone.0122476
4
5 11 Awad AI, Aboud EA. Knowledge, attitude and practice towards antibiotic use
6 among the public in Kuwait. *PLoS One* 2015;**10**:e0117910.
7
8 doi:10.1371/journal.pone.0117910
9
10
11 12 Huang Y, Gu J, Zhang M, *et al.* Knowledge, attitude and practice of
12 antibiotics: a questionnaire study among 2500 Chinese students. *BMC Med*
13 *Educ* 2013;**13**:163. doi:10.1186/1472-6920-13-163
14
15
16 13 Lv B, Zhou Z, Xu G, *et al.* Knowledge, attitudes and practices concerning self-
17 medication with antibiotics among university students in western China. *Trop*
18 *Med Int Heal* 2014;**19**:769–79. doi:10.1111/tmi.12322
19
20
21 14 Teck KC, Ghazi HF, Bin Ahmad MI, *et al.* Knowledge, Attitude, and Practice
22 of Parents Regarding Antibiotic Usage in Treating Children’s Upper
23 Respiratory Tract Infection at Primary Health Clinic in Kuala Lumpur,
24 Malaysia. *Heal Serv Res Manag Epidemiol* 2016;**3**:233339281664372.
25
26 doi:10.1177/2333392816643720
27
28
29 15 Hall MA, Zheng B, Dugan E, *et al.* Measuring Patients’ Trust in their Primary
30 Care Providers. *Med Care Res Rev* 2002;**59**:293–318.
31
32 doi:10.1177/1077558702059003004
33
34
35 16 Performance Standards for Antimicrobial Susceptibility Testing An
36 informational supplement for global application developed through the Clinical
37 and Laboratory Standards Institute.
38
39 <http://ljzx.cqrmhospital.com/upfiles/201601/20160112155335884.pdf>
40
41 (accessed 12 Apr 2018).
42
43
44 17 Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: a new genome assembly
45 algorithm and its applications to single-cell sequencing. *J Comput Biol*
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 2012;**19**:455–77. doi:10.1089/cmb.2012.0021
- 18 Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification
using exact alignments. *Genome Biol* 2014;**15**:R46. doi:10.1186/gb-2014-15-3-
r46
- 19 Madden T. Chapter 16. The BLAST Sequence Analysis Tool.
https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf
(accessed 20 Apr 2018).
- 20 Inouye M, Dashnow H, Raven L-A, *et al.* SRST2: Rapid genomic surveillance
for public health and hospital microbiology labs. *Genome Med* 2014;**6**:90.
doi:10.1186/s13073-014-0090-6
- 21 R Core Team. A language and environment for statistical computing. 2017.
- 22 Young BE, Lye DC, Krishnan P, *et al.* A prospective observational study of the
prevalence and risk factors for colonization by antibiotic resistant bacteria in
patients at admission to hospital in Singapore. *BMC Infect Dis* 2014;**14**:298.
doi:10.1186/1471-2334-14-298
- 23 McNulty CAM, Lecky DM, Xu-McCrae L, *et al.* CTX-M ESBL-producing
Enterobacteriaceae: estimated prevalence in adults in England in 2014. *J*
Antimicrob Chemother Published Online First: 5 March 2018.
doi:10.1093/jac/dky007
- 24 Tängdén T, Cars O, Melhus A, *et al.* Foreign travel is a major risk factor for
colonization with *Escherichia coli* producing CTX-M-type extended-spectrum
beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob*
Agents Chemother 2010;**54**:3564–8. doi:10.1128/AAC.00220-10
- 25 Woerther P-L, Andremont A, Kantele A. Travel-acquired ESBL-producing
Enterobacteriaceae: impact of colonization at individual and community level.

- 1
2
3 *J Travel Med* 2017;**24**:S29–34. doi:10.1093/jtm/taw101
- 4
5 26 Dautzenberg MJD, Haverkate MR, Bonten MJM, *et al.* Epidemic potential of
6
7 Escherichia coli ST131 and Klebsiella pneumoniae ST258: a systematic review
8
9 and meta-analysis. *BMJ Open* 2016;**6**:e009971. doi:10.1136/bmjopen-2015-
10
11 009971
- 12
13 27 Zhong Y-M, Liu W-E, Liang X-H, *et al.* Emergence and spread of O16-ST131
14
15 and O25b-ST131 clones among faecal CTX-M-producing Escherichia coli in
16
17 healthy individuals in Hunan Province, China. *J Antimicrob Chemother*
18
19 2015;**70**:2223–7. doi:10.1093/jac/dkv114
- 20
21
22 28 Niumsup PR, Tansawai U, Na-udom A, *et al.* Prevalence and risk factors for
23
24 intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai
25
26 community. *Eur J Clin Microbiol Infect Dis* 2018;**37**:69–75.
27
28 doi:10.1007/s10096-017-3102-9
- 29
30
31 29 Pitout JDD, DeVinney R. Escherichia coli ST131: a multidrug-resistant clone
32
33 primed for global domination. *F1000Research* 2017;**6**.
34
35 doi:10.12688/f1000research.10609.1
- 36
37
38 30 Harris PNA, Ben Zakour NL, Roberts LW, *et al.* Whole genome analysis of
39
40 cephalosporin-resistant Escherichia coli from bloodstream infections in
41
42 Australia, New Zealand and Singapore: high prevalence of CMY-2 producers
43
44 and ST131 carrying blaCTX-M-15 and blaCTX-M-27. *J Antimicrob*
45
46 *Chemother* 2018;**73**:634–42. doi:10.1093/jac/dkx466
- 47
48 31 Antibiotic resistance: Multi-country public awareness survey. World Health
49
50 Organization.
51
52 2016.<http://www.who.int/drugresistance/documents/baselinesurvey-nov2015/en>
53
54 / (accessed 25 Mar 2018).
- 55
56
57
58
59
60

- 1
2
3 32 Lee T-H, Wong JG, Lye DC, *et al.* Medical and psychosocial factors associated
4 with antibiotic prescribing in primary care: survey questionnaire and factor
5 analysis. *Br J Gen Pract* 2017;**67**:e168–77. doi:10.3399/bjgp17X688885
6
7
8
9 33 Welschen I, Kuyvenhoven M, Hoes A, *et al.* Antibiotics for acute respiratory
10 tract symptoms: patients' expectations, GPs' management and patient
11 satisfaction. *Fam Pract*;21. doi:10.1093/fampra/cmh303
12
13
14
15 34 Davey P, Pagliari C, Hayes A. The patient's role in the spread and control of
16 bacterial resistance to antibiotics. *Clin Microbiol Infect* 2002;**8**:43–68.
17 doi:10.1046/J.1469-0691.8.S.2.6.X
18
19
20
21 35 McNulty CAM, Cookson BD, Lewis MAO. Education of healthcare
22 professionals and the public. *J Antimicrob Chemother* 2012;**67**:i11–8.
23 doi:10.1093/jac/dks199
24
25
26
27 36 Little P, Stuart B, Francis N, *et al.* Effects of internet-based training on
28 antibiotic prescribing rates for acute respiratory-tract infections: a
29 multinational, cluster, randomised, factorial, controlled trial. *Lancet*
30 2013;**382**:1175–82. doi:10.1016/S0140-6736(13)60994-0
31
32
33
34
35
36
37 37 UK Five Year Antimicrobial Resistance Strategy 2013 to 2018.
38 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/att](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/244058/20130902_UK_5_year_AMR_strategy.pdf)
39 [achment_data/file/244058/20130902_UK_5_year_AMR_strategy.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/244058/20130902_UK_5_year_AMR_strategy.pdf)
40 (accessed 5 Apr 2018).
41
42
43
44
45 38 WHO Global Strategy for Containment of Antimicrobial Resistance WHO
46 Global Strategy for Containment of Antimicrobial Resistance. World Health
47 Organization.
48 http://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf
49 (accessed 5 Apr 2018).
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 39 Antibiotics and antibiotic resistance. NPS MedicineWise.
4
5 2018.[https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-](https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-the-facts)
6
7 the-facts (accessed 10 Apr 2018).
8
9 40 2017 Antibiotic Awareness Week Toolkit for Healthcare Organizations and
10
11 Professionals. [https://ipac-](https://ipac-canada.org/photos/custom/Members/pdf/2017_Antibiotic_Awareness_Toolkit(1).pdf)
12
13 [canada.org/photos/custom/Members/pdf/2017_Antibiotic_Awareness_Toolkit](https://ipac-canada.org/photos/custom/Members/pdf/2017_Antibiotic_Awareness_Toolkit(1).pdf)
14
15 (1).pdf (accessed 10 Apr 2018).
16
17 41 Consumer Updates - Combating Antibiotic Resistance.
18
19 2018.<https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm092810.htm>
20
21 (accessed 10 Apr 2018).
22
23 42 Factsheet for the general public - Antimicrobial resistance.
24
25 [https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-](https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-public)
26
27 public (accessed 10 Apr 2018).
28
29 43 Llewelyn MJ, Fitzpatrick JM, Darwin E, *et al.* The antibiotic course has had its
30
31 day. *BMJ* 2017;;j3418. doi:10.1136/bmj.j3418
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

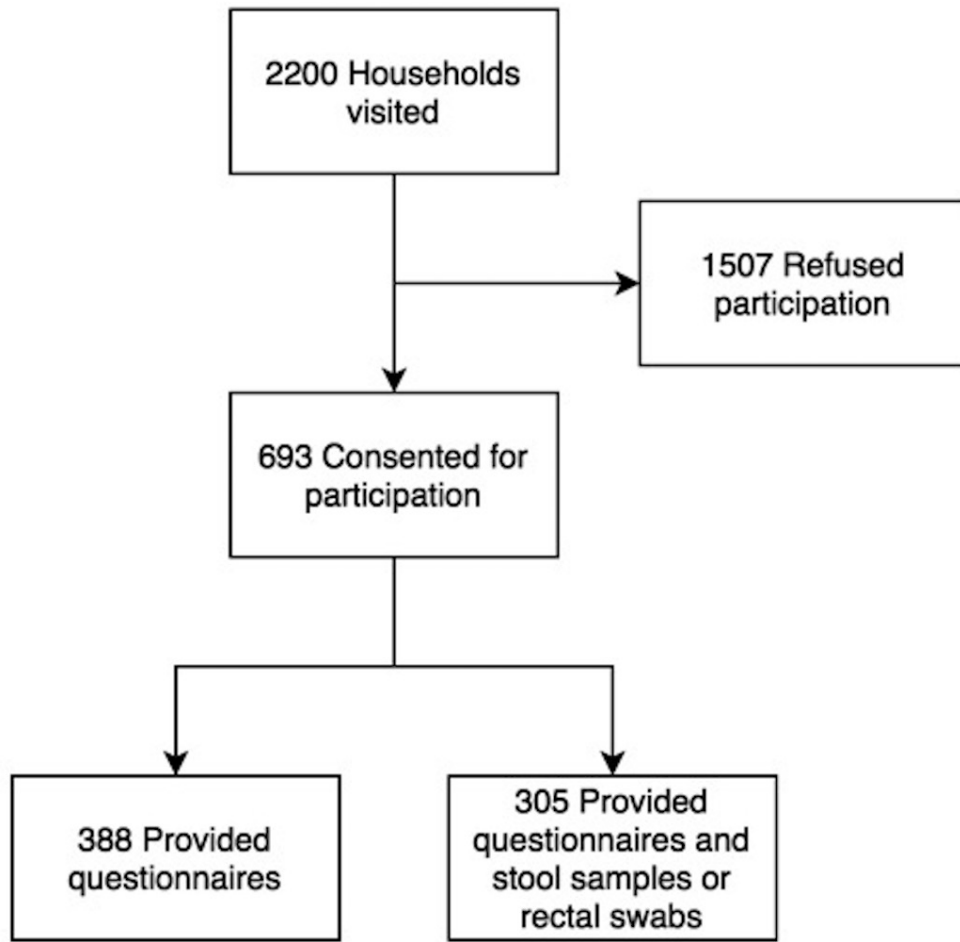


Figure S1. Participant recruitment flowchart

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. Occupation:
- 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

- 1
2
3 5.3 Have you travelled to the following places within the past 6 months? – Yes or
4
5 No
6
7 5.3.1 If yes, which of the following places have you been to? (You may select more
8
9 than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam,
10
11 Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East
12
13 Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or
14
15 North America and/ or Middle East or Others:
16
17
18 5.4 Have you lived anywhere else for more than 1 year? – Yes or No
19
20 6 If yes, did you live in the following areas? (You may select more than 1 option) -
21
22 Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or
23
24 South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea,
25
26 Japan) and/ or Europe and/ or South America and/ or North America and/ or
27
28 Middle East or Others:
29
30
31 7 Medical History
32
33 6.1 Do you have any of the following? (You can choose more than one of the
34
35 following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids,
36
37 Immunosuppressants etc) and/ or Other medical conditions or None of the above
38
39 6.2 When was your last hospitalisation? - Never been hospitalised before or
40
41 Hospitalised before
42
43 6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No
44
45 6.2.2 How long was your stay? Duration:
46
47
48 6.3 Have you used antibiotics before? - Have never used antibiotics before or Used
49
50 antibiotics before
51
52 6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6
53
54
55
56
57
58
59
60

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

1
2
3 or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the
4 rubbish bin or Others:

5
6
7
8 **3. Assessing Alternative Antibiotic Practices**

9
10 **3.1 Have you ever shared antibiotics with someone else? – Yes or No**

11
12
13 **3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes**
14 **or No**

15
16
17
18
19
20
21 **Section 3: Attitude Assessment**

22
23
24 **1. Attitudes Towards Healthcare Provider Prescription**

25
26 **1.1 Sometimes my doctor prioritises what is beneficial for him over my medical**
27 **needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree**

28
29 **1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or**
30 **Agree or Neutral or Disagree or Strongly Disagree**

31
32 **1.3 My doctor is always honest when telling me about all the available treatments for**
33 **my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly**
34 **Disagree**

35
36 **1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or**
37 **Agree or Neutral or Disagree or Strongly Disagree**

38
39
40
41 **2. Attitudes Towards Potential Educational Interventions**

42
43 **2.1 Which of the following sources of medical information do you trust most? -**

44
45 **Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists)**

46
47 **or Family and Friends or Online Medical Sources or Television Programmes and**

48
49 **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? – Viruses or Bacteria or I am not sure
 - 2.2 Which of the following most commonly causes diarrhoea? – Viruses 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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

For peer review only

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)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

For peer review only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	4-5

		<i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	NA
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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.

1
2
3
4 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE
5 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
6 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

For peer review only

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023859.R1
Article Type:	Research
Date Submitted by the Author:	21-Nov-2018
Complete List of Authors:	Mo, Yin; National University Health System, Division of Infectious Diseases, University Medicine Cluster; Mahidol Oxford Tropical Medicine Research Unit Seah, Ivan; National University Singapore Yong Loo Lin School of Medicine Lye, Pei Shi Priscillia ; National University Singapore Yong Loo Lin School of Medicine Kee, Xiang Lee Jamie ; National University Singapore Yong Loo Lin School of Medicine Wong, Kien Yee Michael; National University Singapore Yong Loo Lin School of Medicine Ko, Kwan Ki Karrie ; Singapore General Hospital, Department of Microbiology Ong, Rick Twee-Hee ; National University Singapore Saw Swee Hock School of Public Health Tambyah, Paul; National University Health System, Division of Infectious Diseases, University Medicine Cluster; National University of Singapore, Department of Medicine Cook, Alex R ; National University Singapore Saw Swee Hock School of Public Health
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Public health, Epidemiology
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Antimicrobial resistance, Duration of antibiotic treatment

SCHOLARONE™
Manuscripts

1
2
3 Relating knowledge, attitude and practice of antibiotic use to extended spectrum Beta-
4
5 Lactamase producing *Enterobacteriaceae* carriage – results of a Singapore
6
7
8 Community Survey
9
10
11
12
13

14 Yin MO^{1, 2, 3*}, Ivan SEAH^{4*}, Pei Shi Priscillia LYE⁴, Xiang Lee Jamie KEE⁴, Kien
15
16 Yee Michael WONG⁴, Kwan Ki Karrie KO⁵, Rick Twee-Hee ONG⁶, Paul
17
18 Anantharajah TAMBYAH^{1, 2}, Alex R COOK⁶
19
20
21
22
23
24
25

26 1. Division of Infectious Disease, University Medicine Cluster, National University
27
28 Hospital, 5 Lower Kent Ridge Road Singapore 119074; 2. Department of Medicine,
29
30 National University of Singapore, 21 Lower Kent Ridge Road Singapore 119077; 3.
31
32 Mahidol Oxford Tropical Medicine Research Unit, 420/6 Rajvithi Road,
33
34 Tunphyathai, Bangkok 10400; 4. Yong Loo Lin School of Medicine, 1E Kent Ridge
35
36 Road Singapore 119228; 5. Department of Microbiology, Singapore General
37
38 Hospital, Outram Road Singapore 169608; 6. Saw Swee Hock School of Public
39
40 Health, National University of Singapore, 21 Lower Kent Ridge Road Singapore
41
42 119077
43
44
45
46
47
48

49 * Authors contributed to this work equally.
50
51
52
53

54 Correspondence to: Yin Mo yin_mo@nuhs.edu.sg +65 67795555
55
56
57

58 Word count: 2736
59
60

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

1
2
3 The study was carried out in Clementi Township, a densely populated residential area
4 in the west of Singapore. It comprises 27,142 households with 91,630 residents who
5 are socio-demographically comparable to the general Singapore population in terms
6 of age, gender, ethnicity and housing distribution.[8] The study team returned to non-
7 responding households for up to three times on separate days to maximise the
8 response rate. The first adult above 21 years old in each household who responded to
9 the study team was invited to participate in this cross-sectional study; all consenting
10 individuals undertook a questionnaire, while some additionally consented to provide a
11 rectal swab or stool sample. To calculate the number of samples required to estimate
12 the prevalence of ESBL-PE in the community, we used one-sample Z-test with an
13 estimated prevalence of 50%, a confidence interval of 95% and maximum tolerable
14 error of 10%. This yielded about 100 stool samples. Ethical approval was obtained
15 from National University of Singapore Institutional Review Board (Reference number
16 B-16-245).

17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 **Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake** 39 40 **and health-seeking behaviour**

41 We conducted a questionnaire study to assess the KAP of participants towards
42 antibiotic use. A 40-item questionnaire was developed after performing a thorough
43 literature review of comparable studies.[9–14] This was then validated by a pilot
44 study involving 75 community-dwelling volunteers to ensure fluency and accuracy in
45 question design and language. A team of thirty-three investigators was trained to
46 administer the survey face-to-face, in languages that the participants are fluent in with
47 standardised explanations, to ensure consistency.
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 The questionnaire comprised four main sections. The first covered socio-demographic
4 data and recent antibiotic intake. The second was an assessment of antibiotic
5 consumption practices, in which two hypothetical scenarios of diarrhoea and upper
6 respiratory tract symptoms were presented, and participants were asked if they would
7 visit the doctor should they experience these symptoms for less than 1 week, if they
8 would expect or insist on an antibiotic prescription from the doctor's visit, and if they
9 would seek a second opinion if antibiotics were not prescribed. The third component
10 assessed participants' attitudes and trust towards primary care healthcare providers,
11 and was adapted from a validated questionnaire from Hall *et al.*[15] The last
12 component examined participants' knowledge on AMR. The full questionnaire and
13 grading system can be found in Table S1.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **Bacterial isolation and antibiotic susceptibility testing**

32
33 The study team requested fresh stool samples or rectal swabs from all study
34 participants. The samples of those who consented were collected from the participants
35 within 24 hours of production and stored centrally at 0-4°C prior to microbiological
36 processing. All sample processing was carried out in the Singapore General Hospital
37 Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagarTM*
38 *ESBL* and *CHROMID[®] CARBA SMART (bioMerieux)* media to detect cephalosporin-
39 resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24
40 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and
41 used for subsequent species identification and antibiotic susceptibility testing. Species
42 identification was done by matrix-assisted laser desorption/ionization-time of flight
43 mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 (*bioMerieux*) system.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, ceftiofur, cefepime,
4 amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin,
5 nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin,
6 ertapenem and meropenem were assessed by the disc diffusion method and
7 interpreted according to the Clinical Laboratory Standards Institute (CLSI)
8 criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth
9 generation cephalosporins were identified as potential ESBL producers, while those
10 not susceptible to any carbapenem were identified as potential carbapenemase
11 producers. Potential carbapenemase producers were tested phenotypically for
12 carbapenemase production by modified Hodge test and KPC/MBL and OXA-48
13 Confirm Kit (ROSCO). All potential carbapenemase producers were also subjected to
14 the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM
15 carbapenemase gene sequences.

36 **Whole genome sequencing of ESBL-producing *Enterobacteriaceae***

37
38 DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially
39 ESBL- or carbapenemase- producers, with sequencing libraries for each isolate
40 prepared as per manufacturer's recommendation to be multiplexed sequenced on the
41 Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs,
42 having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads
43 was performed using the SPAdes Genome Assembler.[17] Bacterial species were
44 identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence
45 types (MLSTs) were determined by a customized script utilising BLAST search for
46 identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 resistance owing to the existence of specific gene sequences were performed using
4
5 SRST2.[20]
6
7
8
9

10 **Statistical Analysis**

11
12 Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-
13
14 PE carriage status and presence of specific resistance genes. Dichotomous variables
15
16 are expressed in frequencies and percentages, while continuous variables are in means
17
18 with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's
19
20 exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric
21
22 Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in
23
24 multivariate analyses to identify statistically significant factors that influence and
25
26 determine KAP and ESBL-PE carriage. Covariates that were found to be statistically
27
28 significant in the univariate analyses were included in the multivariate models. All
29
30 tests of significance are performed at $\alpha=5\%$. Statistical analysis was carried out using
31
32 R Version 1.1.383.[21]
33
34
35
36
37
38
39

40 **Patient and Public Involvement**

41
42 A group of 75 community dwellers partnered with us for the design and validation of
43
44 the study questionnaire to ensure clarity and accuracy, production of informational
45
46 material to support recruitment, and evaluation of the burden of the sample collection
47
48 from the patient's perspective. Because there was no clear preference for the sample
49
50 collection methodology, the study team decided to offer both options of rectal swab
51
52 and stool collection to the study participants.
53
54
55
56
57

58 **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

1
2
3 expectation of an antibiotic prescription for common viral infections (OR 0.98,
4
5 95%CI 0.96-1.0).
6
7
8
9

10 A large majority of the community continued to place trust in their primary care
11
12 doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted
13
14 healthcare professionals as their primary source of medical information, over the
15
16 Internet, media and family and friends. There were no significant associations
17
18 between demographic factors and attitude scores in contrast to the differences seen in
19
20 knowledge scores.
21
22
23
24
25

26 In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and
27
28 vomiting), although about half of the participants (294/693, 42.4% for cough and
29
30 runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the
31
32 doctor for common complaints lasting less than 1 week, only 18.5% (average
33
34 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not
35
36 prescribed during the initial visit, very few (average 39/693, 5.6%) reported they
37
38 would insist on antibiotic prescription or seek a second opinion. The only independent
39
40 factor associated with the expectation of an antibiotic prescription was younger age
41
42 (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover
43
44 antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers
45
46 antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing
47
48 with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3%
49
50 (23/693) admitted to having previously shared antibiotics with family members and
51
52 5.5% (38/693) to having taken leftover antibiotics from a previous illness.
53
54
55
56
57
58
59
60

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 *Enterobacteriaceae*

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

	<i>E coli</i> (N=83) N (%)	<i>Klebsiella</i> (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)
Nitrofurantoin	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 mirabilis* (1), *Raoultella ornithinolytica* (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%)

1
2
3 isolates were *E. coli*, of which the most common molecular type was sequence type
4 (ST) 131 (11/71, 15.5%) (Table S5). The most frequently observed ESBL gene was
5 CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27
6 (16/71, 22.5%). *E. coli* ST131 were more resistant to fluoroquinolones than non-
7 ST131 isolates ($p=0.041$). The only significant factor from the questionnaire
8 associated with ESBL-producing *E. coli* ST131 carriage was having more children in
9 the household, but the difference was marginal (mean 0.3 ± 0.7 versus 0.8 ± 1.1 ,
10 $p=0.034$).
11
12
13
14
15
16
17
18
19
20
21
22
23

24 DISCUSSION

25
26 We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy
27 community dwellers in Singapore, twice the rate found in an earlier study in 2014 of
28 patients at an emergency department.[22] Similar rises have been observed
29 globally.[3] Although these figures are lower than the reported prevalence of over
30 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are
31 much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly
32 regulated antibiotic prescription system similar to Europe and the US where only
33 registered medical practitioners are allowed to prescribe antibiotics, and they must be
34 purchased from licensed dispensers. We did not find any association between fecal
35 carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a
36 city-state and overseas travel is very common, making it hard to detect such a
37 relationship when frequent trips to neighbouring countries are made. However, past
38 residency overseas was strongly associated with colonisation, especially those who
39 lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). Distinctions
40 should be made in future studies on overseas travels and AMR carriage in terms of
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 duration and frequency of travel, in addition to destinations. The possibility of
4
5 substantial acquisition of MDRO colonisation and infection through overseas
6
7 exposure[24,25] once again highlights the urgent need for a regional, collaborative
8
9 approach to tackling the problem of AMR.
10
11
12
13

14
15 In addition, we did not find an association between recent antibiotic intake in the past
16
17 6 months and ESBL-PE carriage. This is inconsistent with previous reports showing
18
19 that consumption of certain classes of antibiotics such as beta-lactams and
20
21 fluoroquinolones are risk factors for predispositions to ESBL-PE carriage. [26,27]
22
23 The possible reasons could be due to the relatively small number of participants who
24
25 had recent antibiotic intake (85/305, 27.9%), so we were not able to distinguish the
26
27 specific classes of antibiotics taken by the participants. It is also possible that the
28
29 dominance of a hyperendemic community-associated clone rather than antibiotic
30
31 selection pressure alone contributed to this finding.
32
33
34
35
36
37

38
39 Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131
40
41 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL
42
43 mechanism, echoing the global dissemination of this hyperendemic clone, especially
44
45 in the community.[28] Similar reports showed 11.1% (32/287) in China[29] and 4.1%
46
47 (8/193) in Thailand[30] have been published. The reason for the rapid worldwide
48
49 expansion and long-term persistence of *E. coli* ST131 is thought to be due to
50
51 compensatory mutations within the core genome counterbalancing the fitness cost
52
53 associated with IncF plasmids, thus sustaining its spread even in the absence of direct
54
55 antibiotic selection pressure.[31] These *E. coli* ST131 are not just prevalent colonisers
56
57 but have also associated with invasive bloodstream infections in hospitalized patients
58
59
60

1
2
3 in Australia, New Zealand and Singapore.[32] It will be important to better
4
5 understand the evolutionary ecology and transmission dynamics of this emerging
6
7 clone.
8
9

10
11
12 This study also revealed widespread misconceptions about the utility of antibiotics for
13
14 viral infections, consistent with the findings of a global survey conducted by the
15
16 WHO in 2015.[33] We also found that, the public continues to place trust in their
17
18 primary care doctors and their recommendations. This dependence on physicians is in
19
20 contrast to doctors' perceptions of patient expectations for antibiotic
21
22 prescriptions.[34] This discordance has been previously described and is thought to be
23
24 due to the lack of empowerment of the patient and the erroneous attribution of patient
25
26 satisfaction to antibiotic prescription rather than a focus on better patient-doctor
27
28 communication.[35,36]
29
30
31
32
33

34
35 Engaging and educating both the prescribers and the public may reduce inappropriate
36
37 antibiotic use,[37,38] and has been identified as a key strategy by the WHO and the
38
39 UK to tackle AMR.[39,40] One of the most striking findings of this study is that
40
41 having both the knowledge that antibiotic courses should be completed and not
42
43 having left over antibiotics is independently associated with the carriage of ESBL-PE.
44
45 Though these relationships cannot be viewed as causal given the complexities in the
46
47 emergence and transmission of AMR, there is emerging evidence supporting short
48
49 course antibiotic therapies, even for severe infections such as bacteremia, given the
50
51 collateral damage that antibiotics have on host microbiome.[41] The current WHO
52
53 recommendation remains that full courses of antibiotics should be completed to
54
55 prevent the onset of resistance.[33] Similar messages are advocated in national
56
57
58
59
60

1
2
3 campaigns launched in Australia,[42] the United States[43] and Europe.[44] Given
4
5 that the minimum effective treatment durations have not been determined for many
6
7 infections and that a significant proportion of antibiotic prescriptions are
8
9 inappropriate, the emphasis on completing the course of antibiotics to prevent
10
11 resistance may have to be re-examined.
12
13

14
15
16
17 To our knowledge, this is the first study that explored antibiotic consumption
18
19 behavior with the acquisition of MDRO at a community level. This novel approach
20
21 has the potential to guide clinicians and policy makers in identifying directly
22
23 actionable interventions for the population. The main weakness of our study is that
24
25 the questionnaire data is self-reported and subjected to recall and interviewer biases.
26
27 We minimised these errors by designing specific questions that are carefully
28
29 constructed to maximize accuracy and completeness, and all interviewers were trained
30
31 to adhere to the question and answer format strictly. Further research using antibiotic
32
33 prescription databases can potentially overcome some of the intrinsic biases arising
34
35 from cross-sectional questionnaires.
36
37
38
39
40
41

42 **CONCLUSION**

43
44 There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore,
45
46 especially with *E. coli* ST131 carrying CTX-M. This is correlated with KAP of
47
48 antibiotic use, especially with the practice of finishing full courses of antibiotics, and
49
50 prolonged residency in other parts of Asia. Innovative approaches to control AMR
51
52 that take into account transboundary transmission of resistance and clinical trials to
53
54 determine the appropriate duration of antimicrobial therapy will be critical to control
55
56
57
58
59
60

1
2
3 the emergence of these resistant clones which have contributed significantly to the
4
5 current global antibiotic resistance crisis.
6
7
8
9

10 **CONTRIBUTOR AND GUARANTOR INFORMATION**

11
12 YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the
13
14 study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK
15
16 performed microbiological testing. RTHO planned and conducted genomic
17
18 sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW
19
20 performed data analysis. All participated in the writing of the script, and affirm that
21
22 the manuscript is an honest, accurate, and transparent account of the study being
23
24 reported; that no important aspects of the study have been omitted; and that any
25
26 discrepancies from the study as originally planned have been explained. YM and IS
27
28 accept full responsibility for the work and/or the conduct of the study, had access to
29
30 the data, and controlled the decision to publish. The corresponding author attests that
31
32 all listed authors meet authorship criteria and no others meeting the criteria have been
33
34 omitted.
35
36
37
38
39
40
41

42 **ACKNOWLEDGEMENT**

43
44 The authors thank the study team members, Ang Chen Xiang, Anne Goei Hui Yi,
45
46 Charmaine Loh Hui Yun, Cheong Shao Wei Dominic, Chew Shi Jie, Chong Yvette,
47
48 Choo Hui Min Charlotte, Choo Xin Yi, Daveraj Sivasegaran, Dean Krishen Sethi,
49
50 Joshua Tan Teck Chin, Keith Ching Wei Jie, Khoo Chun Yuet, Krystal Khoo Oon
51
52 Hui, Lai Jieru, Liew Yi Song Terence, Lim Li Liang Joshua, Lok Si Ying Andrea,
53
54 Lynette Sim Pei Shuen, Michelle Sim Yan Lin, Mok Charlene, Ong Yuxuan Daniel,
55
56 Ong Zheng Xuan, Quek Keng Liang, R Krishnapriya, Sophia Ng Shuen Yii, Tan Fang
57
58
59
60

1
2
3 Min Grace, Tan Jian Wei, Tan Pei Min Mabelleline, Tay Yiling Elaine, Tey Min Li,
4
5 Wu Yanlin, Zhou Lingyue, for their contributions in carrying out home visits,
6
7 interviews and sample collections.
8
9

10 11 12 **COPYRIGHT/LICENCE FOR PUBLICATION**

13
14 The Corresponding Author has the right to grant on behalf of all authors and does
15
16 grant on behalf of all authors, a worldwide licence to the Publishers and its licensees
17
18 in the perpetuity, in all forms, formats and media (whether known now or created in
19
20 the future), to i) publish, reproduce, distribute display and store the Contribution, ii)
21
22 translate the Contribution into other languages, create adaptations, reprints, include
23
24 within collections and create summaries, extracts and/or, abstracts of the
25
26 Contribution, iii) create any other derivative work(s) based on the Contribution, iv) to
27
28 exploit all subsidiary rights in the Contribution, v) the inclusion of electronic links
29
30 from the Contribution to third party material where-ever it may be located; and, vi)
31
32 licence any third party to do any or all of the above.
33
34
35
36
37
38
39

40 **FUNDING**

41
42 Data collection was supported from funding from the Infectious Diseases Research
43
44 Fund, National University of Singapore (NUS), and the Saw Swee Hock School of
45
46 Public Health (SSHSPH). RTHO received funding support from the SSHSPH, NUS.
47
48 ARC was supported by the Singapore Population Health Improvement Centre.
49
50
51

52 53 **COMPETING INTERESTS DECLARATION**

54
55 All authors have completed the ICMJE uniform disclosure form at
56
57 www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any
58
59
60

1
2
3 organisations that might have an interest in the submitted work in the previous three
4
5 years; no other relationships or activities that could appear to have influenced the
6
7 submitted work.
8
9

10 11 12 **DATA SHARING**

13
14 The authors commit to making the relevant anonymised patient level data available on
15
16 reasonable request.
17
18

19 20 21 **REFERENCES**

- 22
23
24 1 Tacconelli E, Carrara E, Savoldi A, *et al.* Discovery, research, and
25
26 development of new antibiotics: the WHO priority list of antibiotic-resistant
27
28 bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27.
29
30 doi:10.1016/S1473-3099(17)30753-3
31
32
33 2 Ruppé É, Woerther P-L, Barbier F. Mechanisms of antimicrobial resistance in
34
35 Gram-negative bacilli. *Ann Intensive Care* 2015;**5**:61. doi:10.1186/s13613-015-
36
37 0061-0
38
39
40 3 Karanika S, Karantanos T, Arvanitis M, *et al.* Fecal Colonization With
41
42 Extended-spectrum Beta-lactamase–Producing *Enterobacteriaceae* and Risk
43
44 Factors Among Healthy Individuals: A Systematic Review and Metaanalysis.
45
46 *Clin Infect Dis* 2016;**63**:310–8. doi:10.1093/cid/ciw283
47
48
49 4 Reddy P, Malczynski M, Obias A, *et al.* Screening for Extended-Spectrum -
50
51 Lactamase-Producing *Enterobacteriaceae* among High-Risk Patients and Rates
52
53 of Subsequent Bacteremia. *Clin Infect Dis* 2007;**45**:846–52.
54
55 doi:10.1086/521260
56
57
58 5 Troché G, Toly L-M, Guibert M, *et al.* Detection and Treatment of Antibiotic-
59
60

- 1
2
3 Resistant Bacterial Carriage in a Surgical Intensive Care Unit: A 6-Year
4 Prospective Survey. *Infect Control Hosp Epidemiol* 2005;**26**:161–5.
5
6 doi:10.1086/502521
7
8
9
10 6 Coker RJ, Hunter BM, Rudge JW, *et al.* Emerging infectious diseases in
11 southeast Asia: regional challenges to control. *Lancet* 2011;**377**:599–609.
12
13 doi:10.1016/S0140-6736(10)62004-1
14
15
16
17 7 Zellweger RM, Carrique-Mas J, Limmathurotsakul D, *et al.* A current
18 perspective on antimicrobial resistance in Southeast Asia. *J Antimicrob*
19
20
21
22
23
24
25 8 Singapore Department of Statistics. Singapore Residents by Planning
26 Area/Subzone, 2015. 2015.
27
28
29 9 Pan DST, Huang JH, Lee MHM, *et al.* Knowledge, attitudes and practices
30 towards antibiotic use in upper respiratory tract infections among patients
31 seeking primary health care in Singapore. *BMC Fam Pract* 2016;**17**:148.
32
33
34
35
36
37
38 10 Scaioli G, Gualano MR, Gili R, *et al.* Antibiotic Use: A Cross-Sectional
39 Survey Assessing the Knowledge, Attitudes and Practices amongst Students of
40 a School of Medicine in Italy. *PLoS One* 2015;**10**:e0122476.
41
42
43
44
45
46
47
48
49
50
51
52
53
54 11 Awad AI, Aboud EA. Knowledge, attitude and practice towards antibiotic use
55 among the public in Kuwait. *PLoS One* 2015;**10**:e0117910.
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

- 1
2
3 13 Lv B, Zhou Z, Xu G, *et al.* Knowledge, attitudes and practices concerning self-
4 medication with antibiotics among university students in western China. *Trop*
5 *Med Int Heal* 2014;**19**:769–79. doi:10.1111/tmi.12322
6
7
8
9
10 14 Teck KC, Ghazi HF, Bin Ahmad MI, *et al.* Knowledge, Attitude, and Practice
11 of Parents Regarding Antibiotic Usage in Treating Children’s Upper
12 Respiratory Tract Infection at Primary Health Clinic in Kuala Lumpur,
13 Malaysia. *Heal Serv Res Manag Epidemiol* 2016;**3**:233339281664372.
14 doi:10.1177/2333392816643720
15
16
17
18
19 15 Hall MA, Zheng B, Dugan E, *et al.* Measuring Patients’ Trust in their Primary
20 Care Providers. *Med Care Res Rev* 2002;**59**:293–318.
21 doi:10.1177/1077558702059003004
22
23
24
25
26
27
28 16 Performance Standards for Antimicrobial Susceptibility Testing An
29 informational supplement for global application developed through the Clinical
30 and Laboratory Standards Institute.
31 <http://ljzx.cqrmhospital.com/upfiles/201601/20160112155335884.pdf>
32 (accessed 12 Apr 2018).
33
34
35
36
37
38
39 17 Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: a new genome assembly
40 algorithm and its applications to single-cell sequencing. *J Comput Biol*
41 2012;**19**:455–77. doi:10.1089/cmb.2012.0021
42
43
44
45
46
47 18 Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification
48 using exact alignments. *Genome Biol* 2014;**15**:R46. doi:10.1186/gb-2014-15-3-
49 r46
50
51
52
53 19 Madden T. Chapter 16. The BLAST Sequence Analysis Tool.
54 [https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.p](https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf)
55 [df](https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf) (accessed 20 Apr 2018).
56
57
58
59
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 20 Inouye M, Dashnow H, Raven L-A, *et al.* SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med* 2014;**6**:90. doi:10.1186/s13073-014-0090-6
- 21 R Core Team. A language and environment for statistical computing. 2017.
- 22 Young BE, Lye DC, Krishnan P, *et al.* A prospective observational study of the prevalence and risk factors for colonization by antibiotic resistant bacteria in patients at admission to hospital in Singapore. *BMC Infect Dis* 2014;**14**:298. doi:10.1186/1471-2334-14-298
- 23 McNulty CAM, Lecky DM, Xu-McCrae L, *et al.* CTX-M ESBL-producing Enterobacteriaceae: estimated prevalence in adults in England in 2014. *J Antimicrob Chemother* Published Online First: 5 March 2018. doi:10.1093/jac/dky007
- 24 Tängdén T, Cars O, Melhus A, *et al.* Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* 2010;**54**:3564–8. doi:10.1128/AAC.00220-10
- 25 Woerther P-L, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. *J Travel Med* 2017;**24**:S29–34. doi:10.1093/jtm/taw101
- 26 Augustine MR, Testerman TL, Justo JA, *et al.* Clinical Risk Score for Prediction of Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae in Bloodstream Isolates. *Infect Control Hosp Epidemiol* 2017;**38**:266–72. doi:10.1017/ice.2016.292
- 27 Tumbarello M, Treccarichi EM, Bassetti M, *et al.* Identifying Patients Harboring Extended-Spectrum- β -Lactamase-Producing Enterobacteriaceae on

- 1
2
3 Hospital Admission: Derivation and Validation of a Scoring System.
4
5 *Antimicrob Agents Chemother* 2011;**55**:3485–90. doi:10.1128/AAC.00009-11
6
7
8 28 Dautzenberg MJD, Haverkate MR, Bonten MJM, *et al.* Epidemic potential of
9
10 Escherichia coli ST131 and Klebsiella pneumoniae ST258: a systematic review
11
12 and meta-analysis. *BMJ Open* 2016;**6**:e009971. doi:10.1136/bmjopen-2015-
13
14 009971
15
16
17 29 Zhong Y-M, Liu W-E, Liang X-H, *et al.* Emergence and spread of O16-ST131
18
19 and O25b-ST131 clones among faecal CTX-M-producing Escherichia coli in
20
21 healthy individuals in Hunan Province, China. *J Antimicrob Chemother*
22
23 2015;**70**:2223–7. doi:10.1093/jac/dkv114
24
25
26 30 Niumsup PR, Tansawai U, Na-udom A, *et al.* Prevalence and risk factors for
27
28 intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai
29
30 community. *Eur J Clin Microbiol Infect Dis* 2018;**37**:69–75.
31
32 doi:10.1007/s10096-017-3102-9
33
34
35 31 Pitout JDD, DeVinney R. Escherichia coliST131: a multidrug-resistant clone
36
37 primed for global domination. *F1000Research* 2017;**6**.
38
39 doi:10.12688/f1000research.10609.1
40
41
42 32 Harris PNA, Ben Zakour NL, Roberts LW, *et al.* Whole genome analysis of
43
44 cephalosporin-resistant Escherichia coli from bloodstream infections in
45
46 Australia, New Zealand and Singapore: high prevalence of CMY-2 producers
47
48 and ST131 carrying blaCTX-M-15 and blaCTX-M-27. *J Antimicrob*
49
50 *Chemother* 2018;**73**:634–42. doi:10.1093/jac/dkx466
51
52
53 33 Antibiotic resistance: Multi-country public awareness survey. World Heal.
54
55 Organ.
56
57 2016.<http://www.who.int/drugresistance/documents/baselinesurveynov2015/en>
58
59
60

- 1
2
3 / (accessed 25 Mar 2018).
4
5
6 34 Lee T-H, Wong JG, Lye DC, *et al.* Medical and psychosocial factors associated
7 with antibiotic prescribing in primary care: survey questionnaire and factor
8 analysis. *Br J Gen Pract* 2017;**67**:e168–77. doi:10.3399/bjgp17X688885
9
10
11
12 35 Welschen I, Kuyvenhoven M, Hoes A, *et al.* Antibiotics for acute respiratory
13 tract symptoms: patients' expectations, GPs' management and patient
14 satisfaction. *Fam Pract*; **21**. doi:10.1093/fampra/cmh303
15
16
17
18 36 Davey P, Pagliari C, Hayes A. The patient's role in the spread and control of
19 bacterial resistance to antibiotics. *Clin Microbiol Infect* 2002;**8**:43–68.
20
21
22
23
24
25
26
27 37 McNulty CAM, Cookson BD, Lewis MAO. Education of healthcare
28 professionals and the public. *J Antimicrob Chemother* 2012;**67**:i11–8.
29
30
31
32
33
34 38 Little P, Stuart B, Francis N, *et al.* Effects of internet-based training on
35 antibiotic prescribing rates for acute respiratory-tract infections: a
36 multinational, cluster, randomised, factorial, controlled trial. *Lancet*
37
38
39
40
41
42
43 39 UK Five Year Antimicrobial Resistance Strategy 2013 to 2018.
44
45
46
47
48
49
50
51
52 40 WHO Global Strategy for Containment of Antimicrobial Resistance WHO
53
54
55
56
57
58
59
60

- 1
2
3 41 Llewelyn MJ, Fitzpatrick JM, Darwin E, *et al.* The antibiotic course has had its
4 day. *BMJ* 2017;;j3418. doi:10.1136/bmj.j3418
5
6
7
8 42 Antibiotics and antibiotic resistance. NPS MedicineWise.
9
10 2018.[https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-](https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-the-facts)
11 [the-facts](https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-the-facts) (accessed 10 Apr 2018).
12
13
14 43 Consumer Updates - Combating Antibiotic Resistance.
15
16 2018.<https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm092810.htm>
17
18 (accessed 10 Apr 2018).
19
20
21 44 Factsheet for the general public - Antimicrobial resistance.
22
23 [https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-](https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-public)
24 [public](https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-public) (accessed 10 Apr 2018).
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

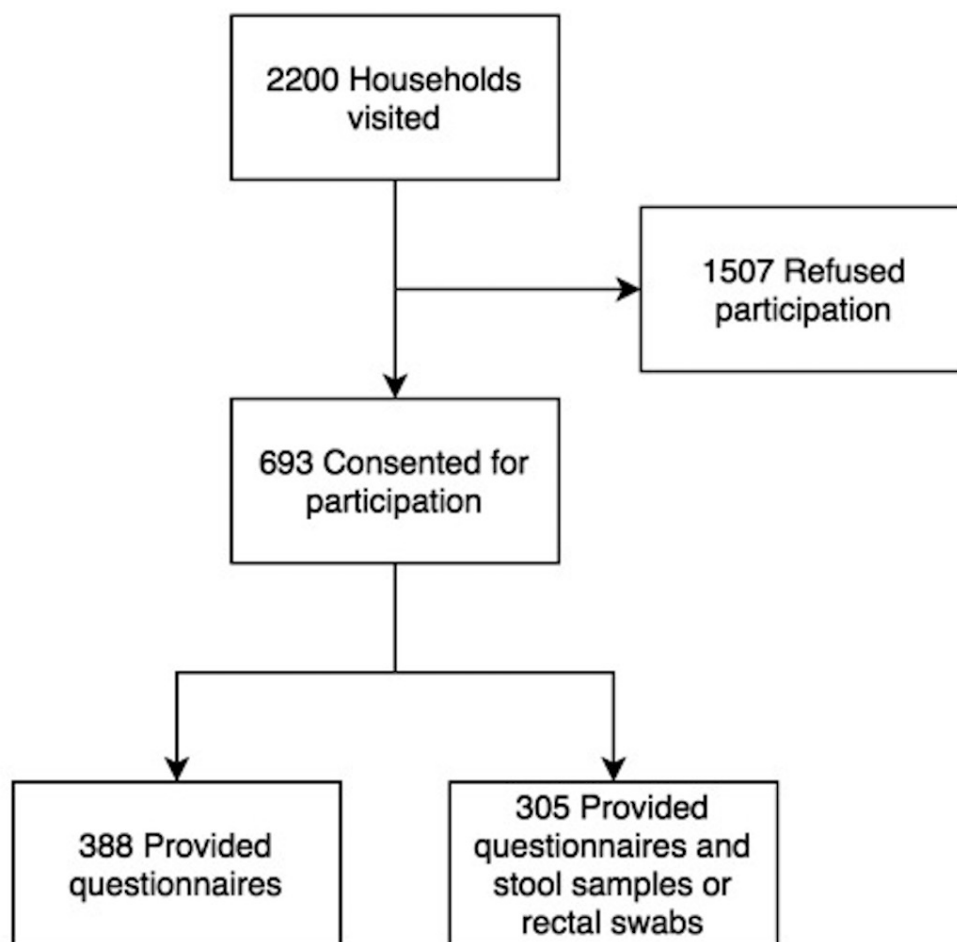


Figure S1. Participant recruitment flowchart

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. Occupation:
 - 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

1
2
3 5.3 Have you travelled to the following places within the past 6 months? – Yes or

4
5 No

6
7
8 5.3.1 If yes, which of the following places have you been to? (You may select more
9
10 than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam,
11
12 Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East
13
14 Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or
15
16 North America and/ or Middle East or Others:

17
18
19 5.4 Have you lived anywhere else for more than 1 year? – Yes or No

20
21
22 6 If yes, did you live in the following areas? (You may select more than 1 option) -
23
24 Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or
25
26 South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea,
27
28 Japan) and/ or Europe and/ or South America and/ or North America and/ or
29
30 Middle East or Others:

31
32
33 7 Medical History

34
35 6.1 Do you have any of the following? (You can choose more than one of the
36
37 following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids,
38
39 Immunosuppressants etc) and/ or Other medical conditions or None of the above

40
41
42 6.2 When was your last hospitalisation? - Never been hospitalised before or
43
44 Hospitalised before

45
46
47 6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

48
49 6.2.2 How long was your stay? Duration:

50
51
52 6.3 Have you used antibiotics before? - Have never used antibiotics before or Used
53
54 antibiotics before

55
56 6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6
57
58
59
60

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

1
2
3 or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the
4 rubbish bin or Others:
5
6
7

8
9 **3. Assessing Alternative Antibiotic Practices**

10
11 3.1 Have you ever shared antibiotics with someone else? – Yes or No
12

13
14 3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes
15
16 or No
17
18
19

20
21
22 **Section 3: Attitude Assessment**

23
24
25 **1. Attitudes Towards Healthcare Provider Prescription**

26
27 1.1 Sometimes my doctor prioritises what is beneficial for him over my medical
28 needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree
29

30
31 1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or
32 Agree or Neutral or Disagree or Strongly Disagree
33
34

35
36 1.3 My doctor is always honest when telling me about all the available treatments for
37 my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly
38 Disagree
39
40
41

42
43 1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or
44 Agree or Neutral or Disagree or Strongly Disagree
45
46
47

48
49 **2. Attitudes Towards Potential Educational Interventions**

50
51 2.1 Which of the following sources of medical information do you trust most? -

52
53 Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists)
54
55 or Family and Friends or Online Medical Sources or Television Programmes and
56
57 Advertisements or Radio Programmes and Advertisements
58
59
60

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

1
2
3 becomes weaker or Your body immunity becomes stronger
4

5 4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am
6

7
8 unsure
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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)

For peer review only

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

	<i>E coli</i>		p-value	
	N=71 (%)			
	ST131 N=11 (%)	Non ST131 N=60 (%)		
Number of resistant genes (mean \pm sd)		1.2 \pm 0.4	1.9 \pm 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)

For peer review only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	4-5

		<i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	NA
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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.

1
2
3
4 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE
5 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
6 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

For peer review only

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023859.R2
Article Type:	Research
Date Submitted by the Author:	23-Jan-2019
Complete List of Authors:	Mo, Yin; National University Health System, Division of Infectious Diseases, University Medicine Cluster; Mahidol Oxford Tropical Medicine Research Unit Seah, Ivan; National University Singapore Yong Loo Lin School of Medicine Lye, Pei Shi Priscillia ; National University Singapore Yong Loo Lin School of Medicine Kee, Xiang Lee Jamie ; National University Singapore Yong Loo Lin School of Medicine Wong, Kien Yee Michael; National University Singapore Yong Loo Lin School of Medicine Ko, Kwan Ki Karrie ; Singapore General Hospital, Department of Microbiology Ong, Rick Twee-Hee ; National University Singapore Saw Swee Hock School of Public Health Tambyah, Paul; National University Health System, Division of Infectious Diseases, University Medicine Cluster; National University of Singapore, Department of Medicine Cook, Alex R ; National University Singapore Saw Swee Hock School of Public Health
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Public health, Epidemiology
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Antimicrobial resistance, Duration of antibiotic treatment

SCHOLARONE™
Manuscripts

1
2
3 Relating knowledge, attitude and practice of antibiotic use to extended spectrum beta-
4 lactamase producing *Enterobacteriaceae* carriage – results of a cross-sectional
5
6
7
8 community Survey
9

10
11
12
13
14 Yin MO^{1, 2, 3*}, Ivan SEAH^{4*}, Pei Shi Priscillia LYE⁴, Xiang Lee Jamie KEE⁴, Kien
15
16
17 Yee Michael WONG⁴, Kwan Ki Karrie KO⁵, Rick Twee-Hee ONG⁶, Paul
18
19 Anantharajah TAMBYAH^{1, 2}, Alex R COOK⁶
20
21
22
23
24
25

26 1. Division of Infectious Disease, University Medicine Cluster, National University
27
28 Hospital, 5 Lower Kent Ridge Road Singapore 119074; 2. Department of Medicine,
29
30 National University of Singapore, 21 Lower Kent Ridge Road Singapore 119077; 3.
31
32 Mahidol Oxford Tropical Medicine Research Unit, 420/6 Rajvithi Road,
33
34 Tunphyathai, Bangkok 10400; 4. Yong Loo Lin School of Medicine, 1E Kent Ridge
35
36 Road Singapore 119228; 5. Department of Microbiology, Singapore General
37
38 Hospital, Outram Road Singapore 169608; 6. Saw Swee Hock School of Public
39
40 Health, National University of Singapore, 21 Lower Kent Ridge Road Singapore
41
42 119077
43
44
45
46
47
48

49 * Authors contributed to this work equally.
50
51
52
53

54 Correspondence to: Yin Mo yin_mo@nuhs.edu.sg +65 67795555
55
56
57

58 Word count: 2736
59
60

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.

1
2
3 (246 words)
4
5
6
7

8 **ARTICLE SUMMARY**

9 **Strengths and limitations of this study**

- 10
11
12
- 13 • We adopted a novel approach of correlating the antibiotic consumption behavior of
14 the patients and general public with asymptomatic multidrug resistance
15 colonisation.
16
17
 - 18 • Based on individual-level data, we found a high prevalence of asymptomatic
19 carriage of extended spectrum beta-lactamase producing *Enterobacteriaceae* in a
20 country with strict antibiotic prescription policies, and this was independently
21 associated with not having left over antibiotics.
22
23
 - 24 • We minimised recall and interviewer biases by designing specific questions that
25 are carefully constructed to maximize accuracy and completeness, and all
26 interviewers were trained to adhere to the question and answer format strictly.
27
28
 - 29 • Given that the minimum effective treatment durations for many infections have not
30 been determined and that a significant proportion of antibiotic prescriptions are
31 inappropriate, the widely accepted message on the necessity to complete antibiotic
32 courses to reduce antibiotic resistance may have to be re-examined.
33
34
 - 35 • Our findings have the potential to guide clinicians and policy makers in addressing
36 this question as part of the effort to control antimicrobial resistance.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3 The study was carried out in Clementi Township, a densely populated residential area
4 in the west of Singapore. It comprises 27,142 households with 91,630 residents who
5 are socio-demographically comparable to the general Singapore population in terms
6 of age, gender, ethnicity and housing distribution.[8] The study team returned to non-
7 responding households for up to three times on separate days to maximise the
8 response rate. The first adult above 21 years old in each household who responded to
9 the study team was invited to participate in this cross-sectional study; all consenting
10 individuals undertook a questionnaire, while some additionally consented to provide a
11 rectal swab or stool sample. To calculate the number of samples required to estimate
12 the prevalence of ESBL-PE in the community, we used one-sample Z-test with an
13 estimated prevalence of 50%, a confidence interval of 95% and maximum tolerable
14 error of 10%. This yielded about 100 stool samples. Ethical approval was obtained
15 from National University of Singapore Institutional Review Board (Reference number
16 B-16-245).

17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 **Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake** 39 40 **and health-seeking behaviour**

41 We conducted a questionnaire study to assess the KAP of participants towards
42 antibiotic use. A 40-item questionnaire was developed after performing a thorough
43 literature review of comparable studies.[9–14] This was then validated by a pilot
44 study involving 75 community-dwelling volunteers to ensure fluency and accuracy in
45 question design and language. A team of thirty-three investigators was trained to
46 administer the survey face-to-face, in languages that the participants are fluent in with
47 standardised explanations, to ensure consistency.
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 The questionnaire comprised four main sections. The first covered socio-demographic
4 data and recent antibiotic intake. The second was an assessment of antibiotic
5 consumption practices, in which two hypothetical scenarios of diarrhoea and upper
6 respiratory tract symptoms were presented, and participants were asked if they would
7 visit the doctor should they experience these symptoms for less than 1 week, if they
8 would expect or insist on an antibiotic prescription from the doctor's visit, and if they
9 would seek a second opinion if antibiotics were not prescribed. The third component
10 assessed participants' attitudes and trust towards primary care healthcare providers,
11 and was adapted from a validated questionnaire from Hall *et al.*[15] The last
12 component examined participants' knowledge on AMR. The full questionnaire and
13 grading system can be found in Table S1.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **Bacterial isolation and antibiotic susceptibility testing**

32
33 The study team requested fresh stool samples or rectal swabs from all study
34 participants. The samples of those who consented were collected from the participants
35 within 24 hours of production and stored centrally at 0-4°C prior to microbiological
36 processing. All sample processing was carried out in the Singapore General Hospital
37 Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagarTM*
38 *ESBL* and *CHROMID[®] CARBA SMART (bioMerieux)* media to detect cephalosporin-
39 resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24
40 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and
41 used for subsequent species identification and antibiotic susceptibility testing. Species
42 identification was done by matrix-assisted laser desorption/ionization-time of flight
43 mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 (*bioMerieux*) system.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, ceftiofur, cefepime,
4 amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin,
5 nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin,
6 ertapenem and meropenem were assessed by the disc diffusion method and
7 interpreted according to the Clinical Laboratory Standards Institute (CLSI)
8 criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth
9 generation cephalosporins were identified as potential ESBL producers, while those
10 not susceptible to any carbapenem were identified as potential carbapenemase
11 producers. Potential carbapenemase producers were tested phenotypically for
12 carbapenemase production by modified Hodge test and KPC/MBL and OXA-48
13 Confirm Kit (ROSCO). All potential carbapenemase producers were also subjected to
14 the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM
15 carbapenemase gene sequences.

36 **Whole genome sequencing of ESBL-producing *Enterobacteriaceae***

37
38 DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially
39 ESBL- or carbapenemase- producers, with sequencing libraries for each isolate
40 prepared as per manufacturer's recommendation to be multiplexed sequenced on the
41 Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs,
42 having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads
43 was performed using the SPAdes Genome Assembler.[17] Bacterial species were
44 identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence
45 types (MLSTs) were determined by a customized script utilising BLAST search for
46 identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 resistance owing to the existence of specific gene sequences were performed using
4
5 SRST2.[20]
6
7
8
9

10 **Statistical Analysis**

11
12 Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-
13
14 PE carriage status and presence of specific resistance genes. Dichotomous variables
15
16 are expressed in frequencies and percentages, while continuous variables are in means
17
18 with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's
19
20 exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric
21
22 Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in
23
24 multivariate analyses to identify statistically significant factors that influence and
25
26 determine KAP and ESBL-PE carriage. Covariates that were found to be statistically
27
28 significant in the univariate analyses were included in the multivariate models. All
29
30 tests of significance are performed at $\alpha=5\%$. Statistical analysis was carried out using
31
32 R Version 1.1.383.[21]
33
34
35
36
37
38
39

40 **Patient and Public Involvement**

41
42 A group of 75 community dwellers partnered with us for the design and validation of
43
44 the study questionnaire to ensure clarity and accuracy, production of informational
45
46 material to support recruitment, and evaluation of the burden of the sample collection
47
48 from the patient's perspective. Because there was no clear preference for the sample
49
50 collection methodology, the study team decided to offer both options of rectal swab
51
52 and stool collection to the study participants.
53
54
55
56
57

58 **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

1
2
3 expectation of an antibiotic prescription for common viral infections (OR 0.98,
4
5 95%CI 0.96-1.0).
6
7
8
9

10 A large majority of the community continued to place trust in their primary care
11
12 doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted
13
14 healthcare professionals as their primary source of medical information, over the
15
16 Internet, media and family and friends. There were no significant associations
17
18 between demographic factors and attitude scores in contrast to the differences seen in
19
20 knowledge scores.
21
22
23
24
25

26 In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and
27
28 vomiting), although about half of the participants (294/693, 42.4% for cough and
29
30 runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the
31
32 doctor for common complaints lasting less than 1 week, only 18.5% (average
33
34 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not
35
36 prescribed during the initial visit, very few (average 39/693, 5.6%) reported they
37
38 would insist on antibiotic prescription or seek a second opinion. The only independent
39
40 factor associated with the expectation of an antibiotic prescription was younger age
41
42 (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover
43
44 antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers
45
46 antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing
47
48 with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3%
49
50 (23/693) admitted to having previously shared antibiotics with family members and
51
52 5.5% (38/693) to having taken leftover antibiotics from a previous illness.
53
54
55
56
57
58
59
60

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 *Enterobacteriaceae*

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 ceftioxin 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

	<i>E coli</i> (N=83) N (%)	<i>Klebsiella</i> (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)
Nitrofurantoin	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 mirabilis* (1), *Raoultella ornithinolytica* (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%)

1
2
3 isolates were *E. coli*, of which the most common molecular type was sequence type
4 (ST) 131 (11/71, 15.5%) (Table S5). The most frequently observed ESBL gene was
5 CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27
6 (16/71, 22.5%). *E. coli* ST131 were more resistant to fluoroquinolones than non-
7 ST131 isolates ($p=0.041$). The only significant factor from the questionnaire
8 associated with ESBL-producing *E. coli* ST131 carriage was having more children in
9 the household, but the difference was marginal (mean 0.3 ± 0.7 versus 0.8 ± 1.1 ,
10 $p=0.034$).
11
12
13
14
15
16
17
18
19
20
21
22
23

24 DISCUSSION

25
26 We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy
27 community dwellers in Singapore, twice the rate found in an earlier study in 2014 of
28 patients at an emergency department.[22] Similar rises have been observed
29 globally.[3] Although these figures are lower than the reported prevalence of over
30 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are
31 much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly
32 regulated antibiotic prescription system similar to Europe and the US where only
33 registered medical practitioners are allowed to prescribe antibiotics, and they must be
34 purchased from licensed dispensers. We did not find any association between fecal
35 carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a
36 city-state and overseas travel is very common, making it hard to detect such a
37 relationship when frequent trips to neighbouring countries are made. However, past
38 residency overseas was strongly associated with colonisation, especially those who
39 lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). Distinctions
40 should be made in future studies on overseas travels and AMR carriage in terms of
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 duration and frequency of travel, in addition to destinations. The possibility of
4
5 substantial acquisition of MDRO colonisation and infection through overseas
6
7 exposure[24,25] once again highlights the urgent need for a regional, collaborative
8
9 approach to tackling the problem of AMR.
10
11
12
13

14
15 In addition, we did not find an association between recent antibiotic intake in the past
16
17 6 months and ESBL-PE carriage. This is inconsistent with previous reports showing
18
19 that consumption of certain classes of antibiotics such as beta-lactams and
20
21 fluoroquinolones are risk factors for predispositions to ESBL-PE carriage. [26,27]
22
23 The possible reasons could be due to the relatively small number of participants who
24
25 had recent antibiotic intake (85/305, 27.9%), so we were not able to distinguish the
26
27 specific classes of antibiotics taken by the participants. It is also possible that the
28
29 dominance of a hyperendemic community-associated clone rather than antibiotic
30
31 selection pressure alone contributed to this finding.
32
33
34
35
36
37

38 Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131
39
40 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL
41
42 mechanism, echoing the global dissemination of this hyperendemic clone, especially
43
44 in the community.[28] Similar reports showed 11.1% (32/287) in China[29] and 4.1%
45
46 (8/193) in Thailand[30] have been published. The reason for the rapid worldwide
47
48 expansion and long-term persistence of *E. coli* ST131 is thought to be due to
49
50 compensatory mutations within the core genome counterbalancing the fitness cost
51
52 associated with IncF plasmids, thus sustaining its spread even in the absence of direct
53
54 antibiotic selection pressure.[31] These *E. coli* ST131 are not just prevalent colonisers
55
56 but have also associated with invasive bloodstream infections in hospitalized patients
57
58
59
60

1
2
3 in Australia, New Zealand and Singapore.[32] It will be important to better
4
5 understand the evolutionary ecology and transmission dynamics of this emerging
6
7 clone.
8
9

10
11
12 This study also revealed widespread misconceptions about the utility of antibiotics for
13
14 viral infections, consistent with the findings of a global survey conducted by the
15
16 WHO in 2015.[33] We also found that, the public continues to place trust in their
17
18 primary care doctors and their recommendations. This dependence on physicians is in
19
20 contrast to doctors' perceptions of patient expectations for antibiotic
21
22 prescriptions.[34] This discordance has been previously described and is thought to be
23
24 due to the lack of empowerment of the patient and the erroneous attribution of patient
25
26 satisfaction to antibiotic prescription rather than a focus on better patient-doctor
27
28 communication.[35,36]
29
30
31
32
33

34
35 Engaging and educating both the prescribers and the public may reduce inappropriate
36
37 antibiotic use,[37,38] and has been identified as a key strategy by the WHO and the
38
39 UK to tackle AMR.[39,40] One of the most striking findings of this study is that
40
41 having both the knowledge that antibiotic courses should be completed and not
42
43 having left over antibiotics is independently associated with the carriage of ESBL-PE.
44
45 Though these relationships cannot be viewed as causal given the complexities in the
46
47 emergence and transmission of AMR, there is emerging evidence supporting short
48
49 course antibiotic therapies, even for severe infections such as bacteremia, given the
50
51 collateral damage that antibiotics have on host microbiome.[41] The current WHO
52
53 recommendation remains that full courses of antibiotics should be completed to
54
55 prevent the onset of resistance.[33] Similar messages are advocated in national
56
57
58
59
60

1
2
3 campaigns launched in Australia,[42] the United States[43] and Europe.[44] Given
4
5 that the minimum effective treatment durations have not been determined for many
6
7 infections and that a significant proportion of antibiotic prescriptions are
8
9 inappropriate, the emphasis on completing the course of antibiotics to prevent
10
11 resistance may have to be re-examined.
12
13

14
15
16
17 To our knowledge, this is the first study that explored antibiotic consumption
18
19 behavior with the acquisition of MDRO at a community level. This novel approach
20
21 has the potential to guide clinicians and policy makers in identifying directly
22
23 actionable interventions for the population. The main weakness of our study is that
24
25 the questionnaire data is self-reported and subjected to recall and interviewer biases.
26
27 We minimised these errors by designing specific questions that are carefully
28
29 constructed to maximize accuracy and completeness, and all interviewers were trained
30
31 to adhere to the question and answer format strictly. Further research using antibiotic
32
33 prescription databases can potentially overcome some of the intrinsic biases arising
34
35 from cross-sectional questionnaires.
36
37
38
39
40
41

42 **CONCLUSION**

43
44 There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore,
45
46 especially with *E. coli* ST131 carrying CTX-M. This is correlated with KAP of
47
48 antibiotic use, especially with the practice of finishing full courses of antibiotics, and
49
50 prolonged residency in other parts of Asia. Innovative approaches to control AMR
51
52 that take into account transboundary transmission of resistance and clinical trials to
53
54 determine the appropriate duration of antimicrobial therapy will be critical to control
55
56
57
58
59
60

1
2
3 the emergence of these resistant clones which have contributed significantly to the
4
5 current global antibiotic resistance crisis.
6
7
8
9

10 **CONTRIBUTOR AND GUARANTOR INFORMATION**

11
12 YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the
13
14 study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK
15
16 performed microbiological testing. RTHO planned and conducted genomic
17
18 sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW
19
20 performed data analysis. All participated in the writing of the script, and affirm that
21
22 the manuscript is an honest, accurate, and transparent account of the study being
23
24 reported; that no important aspects of the study have been omitted; and that any
25
26 discrepancies from the study as originally planned have been explained. YM and IS
27
28 accept full responsibility for the work and/or the conduct of the study, had access to
29
30 the data, and controlled the decision to publish. The corresponding author attests that
31
32 all listed authors meet authorship criteria and no others meeting the criteria have been
33
34 omitted.
35
36
37
38
39
40
41
42

43 **ACKNOWLEDGEMENT**

44 The authors thank the study team members, Ang Chen Xiang, Anne Goei Hui Yi,
45
46 Charmaine Loh Hui Yun, Cheong Shao Wei Dominic, Chew Shi Jie, Chong Yvette,
47
48 Choo Hui Min Charlotte, Choo Xin Yi, Daveraj Sivasegaran, Dean Krishen Sethi,
49
50 Joshua Tan Teck Chin, Keith Ching Wei Jie, Khoo Chun Yuet, Krystal Khoo Oon
51
52 Hui, Lai Jieru, Liew Yi Song Terence, Lim Li Liang Joshua, Lok Si Ying Andrea,
53
54 Lynette Sim Pei Shuen, Michelle Sim Yan Lin, Mok Charlene, Ong Yuxuan Daniel,
55
56 Ong Zheng Xuan, Quek Keng Liang, R Krishnapriya, Sophia Ng Shuen Yii, Tan Fang
57
58
59
60

1
2
3 Min Grace, Tan Jian Wei, Tan Pei Min Mabelleline, Tay Yiling Elaine, Tey Min Li,
4
5 Wu Yanlin, Zhou Lingyue, for their contributions in carrying out home visits,
6
7 interviews and sample collections.
8
9

10 11 12 **COPYRIGHT/LICENCE FOR PUBLICATION**

13
14 The Corresponding Author has the right to grant on behalf of all authors and does
15
16 grant on behalf of all authors, a worldwide licence to the Publishers and its licensees
17
18 in the perpetuity, in all forms, formats and media (whether known now or created in
19
20 the future), to i) publish, reproduce, distribute display and store the Contribution, ii)
21
22 translate the Contribution into other languages, create adaptations, reprints, include
23
24 within collections and create summaries, extracts and/or, abstracts of the
25
26 Contribution, iii) create any other derivative work(s) based on the Contribution, iv) to
27
28 exploit all subsidiary rights in the Contribution, v) the inclusion of electronic links
29
30 from the Contribution to third party material where-ever it may be located; and, vi)
31
32 licence any third party to do any or all of the above.
33
34
35
36
37
38
39

40 **FUNDING**

41
42 Data collection was supported from funding from the Infectious Diseases Research
43
44 Fund, National University of Singapore (NUS), and the Saw Swee Hock School of
45
46 Public Health (SSHSPH). RTHO received funding support from the SSHSPH, NUS.
47
48 ARC was supported by the Singapore Ministry of Health's National Medical
49
50 Research Council under the Centre Grant Programme - Singapore Population Health
51
52 Improvement Centre (NMRC/CG/C026/2017_NUHS).
53
54
55
56
57

58 **COMPETING INTERESTS DECLARATION**

1
2
3 All authors have completed the ICMJE uniform disclosure form at
4
5 www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any
6
7 organisations that might have an interest in the submitted work in the previous three
8
9 years; no other relationships or activities that could appear to have influenced the
10
11 submitted work.
12
13
14
15
16

17 DATA SHARING

18
19 The authors commit to making the relevant anonymised patient level data available on
20
21 reasonable request.
22
23
24
25

26 REFERENCES

- 27
28
29 1 Tacconelli E, Carrara E, Savoldi A, *et al.* Discovery, research, and
30
31 development of new antibiotics: the WHO priority list of antibiotic-resistant
32
33 bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27.
34
35 doi:10.1016/S1473-3099(17)30753-3
36
37
38 2 Ruppé É, Woerther P-L, Barbier F. Mechanisms of antimicrobial resistance in
39
40 Gram-negative bacilli. *Ann Intensive Care* 2015;**5**:61. doi:10.1186/s13613-015-
41
42 0061-0
43
44
45 3 Karanika S, Karantanos T, Arvanitis M, *et al.* Fecal Colonization With
46
47 Extended-spectrum Beta-lactamase–Producing *Enterobacteriaceae* and Risk
48
49 Factors Among Healthy Individuals: A Systematic Review and Metaanalysis.
50
51 *Clin Infect Dis* 2016;**63**:310–8. doi:10.1093/cid/ciw283
52
53
54 4 Reddy P, Malczynski M, Obias A, *et al.* Screening for Extended-Spectrum -
55
56 Lactamase-Producing *Enterobacteriaceae* among High-Risk Patients and Rates
57
58 of Subsequent Bacteremia. *Clin Infect Dis* 2007;**45**:846–52.
59
60

- 1
2
3 doi:10.1086/521260
4
5
6 5 Troché G, Toly L-M, Guibert M, *et al.* Detection and Treatment of Antibiotic-
7
8 Resistant Bacterial Carriage in a Surgical Intensive Care Unit: A 6-Year
9
10 Prospective Survey. *Infect Control Hosp Epidemiol* 2005;**26**:161–5.
11
12 doi:10.1086/502521
13
14
15 6 Coker RJ, Hunter BM, Rudge JW, *et al.* Emerging infectious diseases in
16
17 southeast Asia: regional challenges to control. *Lancet* 2011;**377**:599–609.
18
19 doi:10.1016/S0140-6736(10)62004-1
20
21
22 7 Zellweger RM, Carrique-Mas J, Limmathurotsakul D, *et al.* A current
23
24 perspective on antimicrobial resistance in Southeast Asia. *J Antimicrob*
25
26 *Chemother* 2017;**72**:2963–72. doi:10.1093/jac/dkx260
27
28
29 8 Singapore Department of Statistics. Singapore Residents by Planning
30
31 Area/Subzone, 2015. 2015.
32
33
34 9 Pan DST, Huang JH, Lee MHM, *et al.* Knowledge, attitudes and practices
35
36 towards antibiotic use in upper respiratory tract infections among patients
37
38 seeking primary health care in Singapore. *BMC Fam Pract* 2016;**17**:148.
39
40 doi:10.1186/s12875-016-0547-3
41
42
43 10 Scaioli G, Gualano MR, Gili R, *et al.* Antibiotic Use: A Cross-Sectional
44
45 Survey Assessing the Knowledge, Attitudes and Practices amongst Students of
46
47 a School of Medicine in Italy. *PLoS One* 2015;**10**:e0122476.
48
49 doi:10.1371/journal.pone.0122476
50
51
52 11 Awad AI, Aboud EA. Knowledge, attitude and practice towards antibiotic use
53
54 among the public in Kuwait. *PLoS One* 2015;**10**:e0117910.
55
56 doi:10.1371/journal.pone.0117910
57
58
59 12 Huang Y, Gu J, Zhang M, *et al.* Knowledge, attitude and practice of
60

- 1
2
3 antibiotics: a questionnaire study among 2500 Chinese students. *BMC Med*
4
5 *Educ* 2013;**13**:163. doi:10.1186/1472-6920-13-163
6
7
8 13 Lv B, Zhou Z, Xu G, *et al.* Knowledge, attitudes and practices concerning self-
9
10 medication with antibiotics among university students in western China. *Trop*
11
12 *Med Int Heal* 2014;**19**:769–79. doi:10.1111/tmi.12322
13
14
15 14 Teck KC, Ghazi HF, Bin Ahmad MI, *et al.* Knowledge, Attitude, and Practice
16
17 of Parents Regarding Antibiotic Usage in Treating Children’s Upper
18
19 Respiratory Tract Infection at Primary Health Clinic in Kuala Lumpur,
20
21 Malaysia. *Heal Serv Res Manag Epidemiol* 2016;**3**:233339281664372.
22
23 doi:10.1177/2333392816643720
24
25
26 15 Hall MA, Zheng B, Dugan E, *et al.* Measuring Patients’ Trust in their Primary
27
28 Care Providers. *Med Care Res Rev* 2002;**59**:293–318.
29
30 doi:10.1177/1077558702059003004
31
32
33 16 Performance Standards for Antimicrobial Susceptibility Testing An
34
35 informational supplement for global application developed through the Clinical
36
37 and Laboratory Standards Institute.
38
39 <http://ljzx.cqrmhospital.com/upfiles/201601/20160112155335884.pdf>
40
41 (accessed 12 Apr 2018).
42
43
44 17 Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: a new genome assembly
45
46 algorithm and its applications to single-cell sequencing. *J Comput Biol*
47
48 2012;**19**:455–77. doi:10.1089/cmb.2012.0021
49
50
51 18 Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification
52
53 using exact alignments. *Genome Biol* 2014;**15**:R46. doi:10.1186/gb-2014-15-3-
54
55 r46
56
57
58 19 Madden T. Chapter 16. The BLAST Sequence Analysis Tool.
59
60

- 1
2
3 [https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.p](https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf)
4 [df](https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf) (accessed 20 Apr 2018).
5
6
7
- 8 20 Inouye M, Dashnow H, Raven L-A, *et al.* SRST2: Rapid genomic surveillance
9 for public health and hospital microbiology labs. *Genome Med* 2014;**6**:90.
10 doi:10.1186/s13073-014-0090-6
11
12
13
14
- 15 21 R Core Team. A language and environment for statistical computing. 2017.
16
- 17 22 Young BE, Lye DC, Krishnan P, *et al.* A prospective observational study of the
18 prevalence and risk factors for colonization by antibiotic resistant bacteria in
19 patients at admission to hospital in Singapore. *BMC Infect Dis* 2014;**14**:298.
20 doi:10.1186/1471-2334-14-298
21
22
23
24
- 25 23 McNulty CAM, Lecky DM, Xu-McCrae L, *et al.* CTX-M ESBL-producing
26 Enterobacteriaceae: estimated prevalence in adults in England in 2014. *J*
27 *Antimicrob Chemother* Published Online First: 5 March 2018.
28 doi:10.1093/jac/dky007
29
30
31
32
33
34
- 35 24 Tängdén T, Cars O, Melhus A, *et al.* Foreign travel is a major risk factor for
36 colonization with *Escherichia coli* producing CTX-M-type extended-spectrum
37 beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob*
38 *Agents Chemother* 2010;**54**:3564–8. doi:10.1128/AAC.00220-10
39
40
41
42
43
44
- 45 25 Woerther P-L, Andremont A, Kantele A. Travel-acquired ESBL-producing
46 Enterobacteriaceae: impact of colonization at individual and community level.
47 *J Travel Med* 2017;**24**:S29–34. doi:10.1093/jtm/taw101
48
49
50
- 51 26 Augustine MR, Testerman TL, Justo JA, *et al.* Clinical Risk Score for
52 Prediction of Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae
53 in Bloodstream Isolates. *Infect Control Hosp Epidemiol* 2017;**38**:266–72.
54
55
56
57
58
59
60

- 1
2
3 27 Tumbarello M, Treccarichi EM, Bassetti M, *et al.* Identifying Patients
4
5 Harboring Extended-Spectrum- β -Lactamase-Producing Enterobacteriaceae on
6
7 Hospital Admission: Derivation and Validation of a Scoring System.
8
9
10 *Antimicrob Agents Chemother* 2011;**55**:3485–90. doi:10.1128/AAC.00009-11
11
12 28 Dautzenberg MJD, Haverkate MR, Bonten MJM, *et al.* Epidemic potential of
13
14 Escherichia coli ST131 and Klebsiella pneumoniae ST258: a systematic review
15
16 and meta-analysis. *BMJ Open* 2016;**6**:e009971. doi:10.1136/bmjopen-2015-
17
18 009971
19
20
21 29 Zhong Y-M, Liu W-E, Liang X-H, *et al.* Emergence and spread of O16-ST131
22
23 and O25b-ST131 clones among faecal CTX-M-producing Escherichia coli in
24
25 healthy individuals in Hunan Province, China. *J Antimicrob Chemother*
26
27 2015;**70**:2223–7. doi:10.1093/jac/dkv114
28
29
30 30 Niumsup PR, Tansawai U, Na-udom A, *et al.* Prevalence and risk factors for
31
32 intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai
33
34 community. *Eur J Clin Microbiol Infect Dis* 2018;**37**:69–75.
35
36 doi:10.1007/s10096-017-3102-9
37
38
39 31 Pitout JDD, DeVinney R. Escherichia coli ST131: a multidrug-resistant clone
40
41 primed for global domination. *F1000Research* 2017;**6**.
42
43 doi:10.12688/f1000research.10609.1
44
45
46 32 Harris PNA, Ben Zakour NL, Roberts LW, *et al.* Whole genome analysis of
47
48 cephalosporin-resistant Escherichia coli from bloodstream infections in
49
50 Australia, New Zealand and Singapore: high prevalence of CMY-2 producers
51
52 and ST131 carrying blaCTX-M-15 and blaCTX-M-27. *J Antimicrob*
53
54 *Chemother* 2018;**73**:634–42. doi:10.1093/jac/dkx466
55
56
57 33 Antibiotic resistance: Multi-country public awareness survey. World Heal.
58
59
60

- 1
2
3 Organ.
4
5 2016.<http://www.who.int/drugresistance/documents/baselinesurveynov2015/en>
6
7 / (accessed 25 Mar 2018).
8
9
- 10 34 Lee T-H, Wong JG, Lye DC, *et al.* Medical and psychosocial factors associated
11 with antibiotic prescribing in primary care: survey questionnaire and factor
12 analysis. *Br J Gen Pract* 2017;**67**:e168–77. doi:10.3399/bjgp17X688885
13
14
- 15 35 Welschen I, Kuyvenhoven M, Hoes A, *et al.* Antibiotics for acute respiratory
16 tract symptoms: patients' expectations, GPs' management and patient
17 satisfaction. *Fam Pract*; **21**. doi:10.1093/fampra/cmh303
18
19
- 20 36 Davey P, Pagliari C, Hayes A. The patient's role in the spread and control of
21 bacterial resistance to antibiotics. *Clin Microbiol Infect* 2002;**8**:43–68.
22 doi:10.1046/J.1469-0691.8.S.2.6.X
23
24
- 25 37 McNulty CAM, Cookson BD, Lewis MAO. Education of healthcare
26 professionals and the public. *J Antimicrob Chemother* 2012;**67**:i11–8.
27 doi:10.1093/jac/dks199
28
29
- 30 38 Little P, Stuart B, Francis N, *et al.* Effects of internet-based training on
31 antibiotic prescribing rates for acute respiratory-tract infections: a
32 multinational, cluster, randomised, factorial, controlled trial. *Lancet*
33 2013;**382**:1175–82. doi:10.1016/S0140-6736(13)60994-0
34
35
- 36 39 UK Five Year Antimicrobial Resistance Strategy 2013 to 2018.
37 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/att](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/244058/20130902_UK_5_year_AMR_strategy.pdf)
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 40 WHO Global Strategy for Containment of Antimicrobial Resistance WHO
Global Strategy for Containment of Antimicrobial Resistance. World Heal.

- 1
2
3 Organ. http://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf
4
5 (accessed 5 Apr 2018).
6
7
8 41 Llewelyn MJ, Fitzpatrick JM, Darwin E, *et al.* The antibiotic course has had its
9
10 day. *BMJ* 2017;;j3418. doi:10.1136/bmj.j3418
11
12 42 Antibiotics and antibiotic resistance. NPS MedicineWise.
13
14 2018.[https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-](https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-the-facts)
15
16 the-facts (accessed 10 Apr 2018).
17
18
19 43 Consumer Updates - Combating Antibiotic Resistance.
20
21 2018.<https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm092810.htm>
22
23 (accessed 10 Apr 2018).
24
25
26 44 Factsheet for the general public - Antimicrobial resistance.
27
28 [https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-](https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-public)
29
30 public (accessed 10 Apr 2018).
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

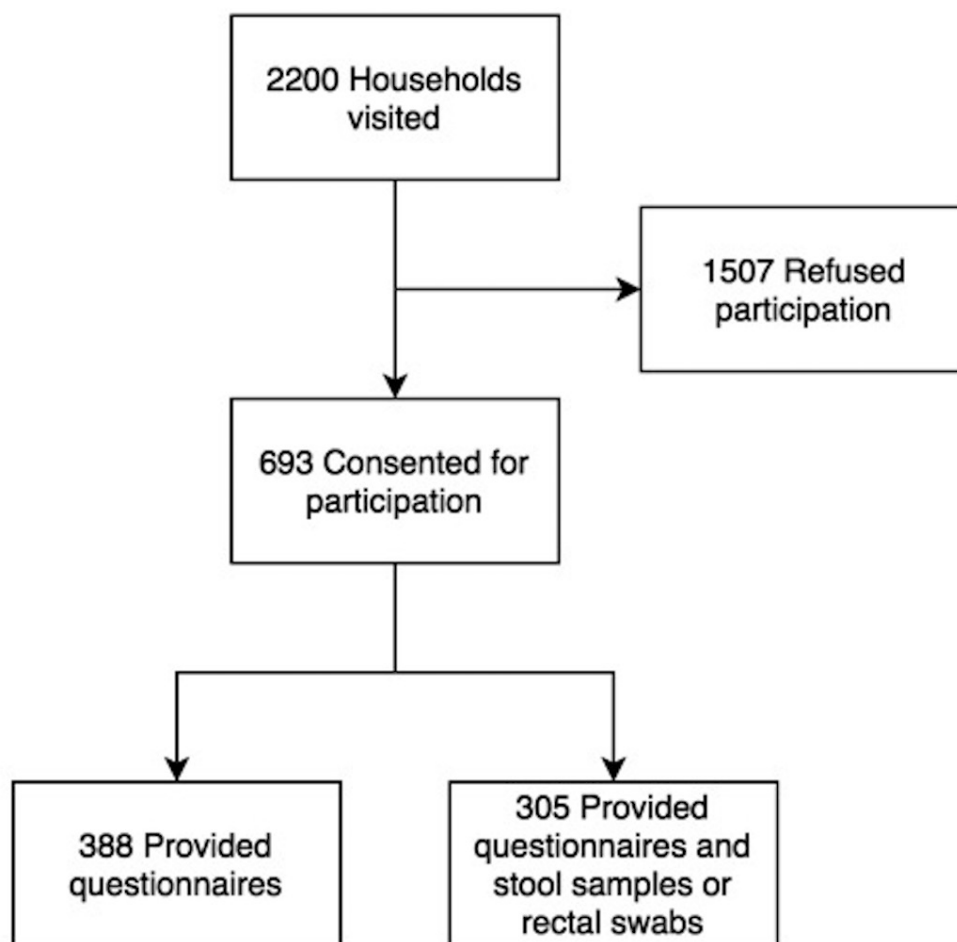


Figure S1. Participant recruitment flowchart

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. Occupation:
 - 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

1
2
3 5.3 Have you travelled to the following places within the past 6 months? – Yes or

4
5 No

6
7
8 5.3.1 If yes, which of the following places have you been to? (You may select more
9
10 than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam,
11
12 Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East
13
14 Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or
15
16 North America and/ or Middle East or Others:

17
18
19 5.4 Have you lived anywhere else for more than 1 year? – Yes or No

20
21
22 6 If yes, did you live in the following areas? (You may select more than 1 option) -
23
24 Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or
25
26 South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea,
27
28 Japan) and/ or Europe and/ or South America and/ or North America and/ or
29
30 Middle East or Others:

31
32
33 7 Medical History

34
35 6.1 Do you have any of the following? (You can choose more than one of the
36
37 following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids,
38
39 Immunosuppressants etc) and/ or Other medical conditions or None of the above

40
41
42 6.2 When was your last hospitalisation? - Never been hospitalised before or
43
44 Hospitalised before

45
46
47 6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

48
49 6.2.2 How long was your stay? Duration:

50
51
52 6.3 Have you used antibiotics before? - Have never used antibiotics before or Used
53
54 antibiotics before

55
56 6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6
57
58
59
60

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

1
2
3 or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the
4 rubbish bin or Others:
5
6

7
8
9 **3. Assessing Alternative Antibiotic Practices**

10
11 **3.1** Have you ever shared antibiotics with someone else? – Yes or No

12
13
14 **3.2** Have you ever taken leftover antibiotics from a previous course of illness? – Yes
15 or No
16
17
18
19

20
21
22
23 **Section 3: Attitude Assessment**

24
25
26 **1. Attitudes Towards Healthcare Provider Prescription**

27
28 **1.1** Sometimes my doctor prioritises what is beneficial for him over my medical
29 needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree
30

31
32 **1.2** My doctor's medical skills are not as good as they should be. – Strongly agree or
33 Agree or Neutral or Disagree or Strongly Disagree
34

35
36 **1.3** My doctor is always honest when telling me about all the available treatments for
37 my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly
38 Disagree
39
40
41

42
43 **1.4** I have no worries about putting my life in my doctor's hands. – Strongly agree or
44 Agree or Neutral or Disagree or Strongly Disagree
45
46
47

48
49 **2. Attitudes Towards Potential Educational Interventions**

50
51 **2.1** Which of the following sources of medical information do you trust most? -

52
53 Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists)
54 or Family and Friends or Online Medical Sources or Television Programmes and
55 Advertisements or Radio Programmes and Advertisements
56
57
58
59
60

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

1
2
3 becomes weaker or Your body immunity becomes stronger
4

5 4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am
6

7
8 unsure
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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%)

1 2 3 4 5 6 7 8 9	2.1.8	What causes antimicrobial resistance? (Open ended)	Inappropriate use of antibiotics	121 (17.5%)
			Wrong or unsure	572 (82.5%)
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34	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%)
35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	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)

For peer review only

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

	<i>E coli</i>			p-value
	N=71 (%)			
	ST131 N=11 (%)	Non ST131 N=60 (%)		
Number of resistant genes (mean \pm sd)		1.2 \pm 0.4	1.9 \pm 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)

For peer review only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	4-5

		<i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	NA
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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.

1
2
3
4 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE
5 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
6 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

For peer review only

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023859.R3
Article Type:	Research
Date Submitted by the Author:	28-Jan-2019
Complete List of Authors:	Mo, Yin; National University Health System, Division of Infectious Diseases, University Medicine Cluster; Mahidol Oxford Tropical Medicine Research Unit Seah, Ivan; National University Singapore Yong Loo Lin School of Medicine Lye, Pei Shi Priscillia ; National University Singapore Yong Loo Lin School of Medicine Kee, Xiang Lee Jamie ; National University Singapore Yong Loo Lin School of Medicine Wong, Kien Yee Michael; National University Singapore Yong Loo Lin School of Medicine Ko, Kwan Ki Karrie ; Singapore General Hospital, Department of Microbiology Ong, Rick Twee-Hee ; National University Singapore Saw Swee Hock School of Public Health Tambyah, Paul; National University Health System, Division of Infectious Diseases, University Medicine Cluster; National University of Singapore, Department of Medicine Cook, Alex R ; National University Singapore Saw Swee Hock School of Public Health
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Public health, Epidemiology
Keywords:	Extended-spectrum beta-lactamase producing Enterobacteriaceae, Antimicrobial resistance, Duration of antibiotic treatment

SCHOLARONE™
Manuscripts

1
2
3 Relating knowledge, attitude and practice of antibiotic use to extended spectrum beta-
4 lactamase producing *Enterobacteriaceae* carriage – results of a cross-sectional
5
6
7
8 community Survey
9

10
11
12
13
14 Yin MO^{1, 2, 3*}, Ivan SEAH^{4*}, Pei Shi Priscillia LYE⁴, Xiang Lee Jamie KEE⁴, Kien
15
16
17 Yee Michael WONG⁴, Kwan Ki Karrie KO⁵, Rick Twee-Hee ONG⁶, Paul
18
19 Anantharajah TAMBYAH^{1, 2}, Alex R COOK⁶
20
21
22
23
24
25

26 1. Division of Infectious Disease, University Medicine Cluster, National University
27
28 Hospital, 5 Lower Kent Ridge Road Singapore 119074; 2. Department of Medicine,
29
30 National University of Singapore, 21 Lower Kent Ridge Road Singapore 119077; 3.
31
32 Mahidol Oxford Tropical Medicine Research Unit, 420/6 Rajvithi Road,
33
34 Tunphyathai, Bangkok 10400; 4. Yong Loo Lin School of Medicine, 1E Kent Ridge
35
36 Road Singapore 119228; 5. Department of Microbiology, Singapore General
37
38 Hospital, Outram Road Singapore 169608; 6. Saw Swee Hock School of Public
39
40 Health, National University of Singapore, 21 Lower Kent Ridge Road Singapore
41
42 119077
43
44
45
46
47
48

49 * Authors contributed to this work equally.
50
51
52
53

54 Correspondence to: Yin Mo yin_mo@nuhs.edu.sg +65 67795555
55
56
57

58 Word count: 2736
59
60

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.

1
2
3 (246 words)
4
5
6
7

8 **ARTICLE SUMMARY**

9 **Strengths and limitations of this study**

- 10
11
12
- 13 • Based on individual-level data, we adopted a novel approach of correlating
14 knowledge, attitude and practice of antibiotic use with asymptomatic carriage of
15 extended spectrum beta-lactamase producing *Enterobacteriaceae* to identify
16 modifiable factors to mitigate antimicrobial resistance in the community.
17
18
 - 19 • We randomly sampled a large number of households in the community
20 representative of the Singaporean general public in terms of demographics and
21 socioeconomic status.
22
23
 - 24 • Extended spectrum beta-lactamase producing *Enterobacteriaceae* were confirmed
25 with both phenotypic antibiotic susceptibilities and whole genome sequencing.
26
27
 - 28 • We minimised recall and interviewer biases by designing specific questions that
29 are carefully constructed to maximize accuracy and completeness, and all
30 interviewers were trained to adhere to the question and answer format strictly.
31
32
 - 33 • Correlations found in the study cannot be viewed as causal given the complexities
34 in the emergence and transmission of AMR.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3 The study was carried out in Clementi Township, a densely populated residential area
4 in the west of Singapore. It comprises 27,142 households with 91,630 residents who
5 are socio-demographically comparable to the general Singapore population in terms
6 of age, gender, ethnicity and housing distribution.[8] The study team returned to non-
7 responding households for up to three times on separate days to maximise the
8 response rate. The first adult above 21 years old in each household who responded to
9 the study team was invited to participate in this cross-sectional study; all consenting
10 individuals undertook a questionnaire, while some additionally consented to provide a
11 rectal swab or stool sample. To calculate the number of samples required to estimate
12 the prevalence of ESBL-PE in the community, we used one-sample Z-test with an
13 estimated prevalence of 50%, a confidence interval of 95% and maximum tolerable
14 error of 10%. This yielded about 100 stool samples. Ethical approval was obtained
15 from National University of Singapore Institutional Review Board (Reference number
16 B-16-245).

37 **Questionnaire on knowledge, attitudes and practices (KAP) on antibiotic intake** 38 **and health-seeking behaviour**

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

1
2
3 The questionnaire comprised four main sections. The first covered socio-demographic
4 data and recent antibiotic intake. The second was an assessment of antibiotic
5 consumption practices, in which two hypothetical scenarios of diarrhoea and upper
6 respiratory tract symptoms were presented, and participants were asked if they would
7 visit the doctor should they experience these symptoms for less than 1 week, if they
8 would expect or insist on an antibiotic prescription from the doctor's visit, and if they
9 would seek a second opinion if antibiotics were not prescribed. The third component
10 assessed participants' attitudes and trust towards primary care healthcare providers,
11 and was adapted from a validated questionnaire from Hall *et al.*[15] The last
12 component examined participants' knowledge on AMR. The full questionnaire and
13 grading system can be found in Table S1.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **Bacterial isolation and antibiotic susceptibility testing**

32
33 The study team requested fresh stool samples or rectal swabs from all study
34 participants. The samples of those who consented were collected from the participants
35 within 24 hours of production and stored centrally at 0-4°C prior to microbiological
36 processing. All sample processing was carried out in the Singapore General Hospital
37 Diagnostic Bacteriology Laboratory. Samples were inoculated onto *CHROMagarTM*
38 *ESBL* and *CHROMID[®] CARBA SMART (bioMerieux)* media to detect cephalosporin-
39 resistant and carbapenem-resistant Gram-negative bacteria, respectively. After 24
40 hours of incubation, growing colonies were sub-cultured onto sheep blood agar and
41 used for subsequent species identification and antibiotic susceptibility testing. Species
42 identification was done by matrix-assisted laser desorption/ionization-time of flight
43 mass spectrometry (MALDI-TOF MS) (Bruker) and the Vitek-2 (*bioMerieux*) system.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Antibiotic susceptibilities to ampicillin, cefazolin, ceftriaxone, ceftiofur, cefepime,
4 amoxicillin-clavulanic acid, piperacillin-tazobactam, aztreonam, amikacin,
5 nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, ciprofloxacin, fosfomycin,
6 ertapenem and meropenem were assessed by the disc diffusion method and
7 interpreted according to the Clinical Laboratory Standards Institute (CLSI)
8 criteria.[16] *Enterobacteriaceae* isolates that were not susceptible to third/ fourth
9 generation cephalosporins were identified as potential ESBL producers, while those
10 not susceptible to any carbapenem were identified as potential carbapenemase
11 producers. Potential carbapenemase producers were tested phenotypically for
12 carbapenemase production by modified Hodge test and KPC/MBL and OXA-48
13 Confirm Kit (ROSCO). All potential carbapenemase producers were also subjected to
14 the Xpert® Carba-R test (Cepheid) targeting KPC, NDM, OXA-48 like, IMP and VIM
15 carbapenemase gene sequences.

36 **Whole genome sequencing of ESBL-producing *Enterobacteriaceae***

37
38 DNA extraction was performed for all *Enterobacteriaceae* isolates that are potentially
39 ESBL- or carbapenemase- producers, with sequencing libraries for each isolate
40 prepared as per manufacturer's recommendation to be multiplexed sequenced on the
41 Illumina HiSEQ platform generating paired-end sequence reads of 2x150 basepairs,
42 having a data throughput of 1GB per isolate. De-novo assembly of the Illumina reads
43 was performed using the SPAdes Genome Assembler.[17] Bacterial species were
44 identified using Kraken,[18] comparing with phenotypic results. Multi-locus sequence
45 types (MLSTs) were determined by a customized script utilising BLAST search for
46 identification of genotypes at each loci.[19] Genotypic prediction of antimicrobial
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 resistance owing to the existence of specific gene sequences were performed using
4
5 SRST2.[20]
6
7
8
9

10 **Statistical Analysis**

11
12 Univariate descriptive analyses are presented for socio-demographics, ESBL-PE or C-
13
14 PE carriage status and presence of specific resistance genes. Dichotomous variables
15
16 are expressed in frequencies and percentages, while continuous variables are in means
17
18 with standard deviation (SD). Categorical variables are compared with χ^2 and Fisher's
19
20 exact tests and continuous variables with unpaired, 2-tailed t tests or nonparametric
21
22 Wilcoxon rank sum tests as appropriate. Linear and logistic regressions are used in
23
24 multivariate analyses to identify statistically significant factors that influence and
25
26 determine KAP and ESBL-PE carriage. Covariates that were found to be statistically
27
28 significant in the univariate analyses were included in the multivariate models. All
29
30 tests of significance are performed at $\alpha=5\%$. Statistical analysis was carried out using
31
32 R Version 1.1.383.[21]
33
34
35
36
37
38
39

40 **Patient and Public Involvement**

41
42 A group of 75 community dwellers partnered with us for the design and validation of
43
44 the study questionnaire to ensure clarity and accuracy, production of informational
45
46 material to support recruitment, and evaluation of the burden of the sample collection
47
48 from the patient's perspective. Because there was no clear preference for the sample
49
50 collection methodology, the study team decided to offer both options of rectal swab
51
52 and stool collection to the study participants.
53
54
55
56
57

58 **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

1
2
3 expectation of an antibiotic prescription for common viral infections (OR 0.98,
4
5 95%CI 0.96-1.0).
6
7
8
9

10 A large majority of the community continued to place trust in their primary care
11
12 doctors (Table S3). Most strikingly, 627 participants (627/693, 90.6%) trusted
13
14 healthcare professionals as their primary source of medical information, over the
15
16 Internet, media and family and friends. There were no significant associations
17
18 between demographic factors and attitude scores in contrast to the differences seen in
19
20 knowledge scores.
21
22
23
24
25

26 In the two scenarios (of having an upper-respiratory tract infection or diarrhoea and
27
28 vomiting), although about half of the participants (294/693, 42.4% for cough and
29
30 runny nose, 414/693, 59.7% for diarrhoea and vomiting) envisioned visiting the
31
32 doctor for common complaints lasting less than 1 week, only 18.5% (average
33
34 128/693) expected an antibiotic prescription (Table S4). Were antibiotics not
35
36 prescribed during the initial visit, very few (average 39/693, 5.6%) reported they
37
38 would insist on antibiotic prescription or seek a second opinion. The only independent
39
40 factor associated with the expectation of an antibiotic prescription was younger age
41
42 (OR 0.98, 95%CI 0.97- 0.99) in multivariate logistic analysis. In dealing with leftover
43
44 antibiotics, the majority 68.7% (476/693) declared that they do not have leftovers
45
46 antibiotics; others reported keeping them for future use (60/693, 8.7%) or disposing
47
48 with solid waste (130/693, 18.8%) or down the drain (8/693, 1.2%). Only 3.3%
49
50 (23/693) admitted to having previously shared antibiotics with family members and
51
52 5.5% (38/693) to having taken leftover antibiotics from a previous illness.
53
54
55
56
57
58
59
60

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 *Enterobacteriaceae*

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

	<i>E coli</i> (N=83) N (%)	<i>Klebsiella</i> (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)
Nitrofurantoin	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 mirabilis* (1), *Raoultella ornithinolytica* (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%)

1
2
3 isolates were *E. coli*, of which the most common molecular type was sequence type
4 (ST) 131 (11/71, 15.5%) (Table S5). The most frequently observed ESBL gene was
5 CTX-M (62/80, 77.5%), especially CTX-M-15 (21/71, 29.6%) and CTX-M-27
6 (16/71, 22.5%). *E. coli* ST131 were more resistant to fluoroquinolones than non-
7 ST131 isolates ($p=0.041$). The only significant factor from the questionnaire
8 associated with ESBL-producing *E. coli* ST131 carriage was having more children in
9 the household, but the difference was marginal (mean 0.3 ± 0.7 versus 0.8 ± 1.1 ,
10 $p=0.034$).
11
12
13
14
15
16
17
18
19
20
21
22
23

24 DISCUSSION

25
26 We found a significant burden of ESBL-PE carriage (80/305, 26.2%) among healthy
27 community dwellers in Singapore, twice the rate found in an earlier study in 2014 of
28 patients at an emergency department.[22] Similar rises have been observed
29 globally.[3] Although these figures are lower than the reported prevalence of over
30 40% fecal carriage with ESBL-PE elsewhere in South and South East Asia, they are
31 much higher than the 1.5-3% observed in the US and UK.[3] Singapore has a tightly
32 regulated antibiotic prescription system similar to Europe and the US where only
33 registered medical practitioners are allowed to prescribe antibiotics, and they must be
34 purchased from licensed dispensers. We did not find any association between fecal
35 carriage of ESBL-PE and short-term travel, unlike other studies.[23] Singapore is a
36 city-state and overseas travel is very common, making it hard to detect such a
37 relationship when frequent trips to neighbouring countries are made. However, past
38 residency overseas was strongly associated with colonisation, especially those who
39 lived elsewhere in South or South East Asia (OR 3.3, 95%CI 1.6- 6.9). Distinctions
40 should be made in future studies on overseas travels and AMR carriage in terms of
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 duration and frequency of travel, in addition to destinations. The possibility of
4
5 substantial acquisition of MDRO colonisation and infection through overseas
6
7 exposure[24,25] once again highlights the urgent need for a regional, collaborative
8
9 approach to tackling the problem of AMR.
10
11
12
13

14
15 In addition, we did not find an association between recent antibiotic intake in the past
16
17 6 months and ESBL-PE carriage. This is inconsistent with previous reports showing
18
19 that consumption of certain classes of antibiotics such as beta-lactams and
20
21 fluoroquinolones are risk factors for predispositions to ESBL-PE carriage. [26,27]
22
23 The possible reasons could be due to the relatively small number of participants who
24
25 had recent antibiotic intake (85/305, 27.9%), so we were not able to distinguish the
26
27 specific classes of antibiotics taken by the participants. It is also possible that the
28
29 dominance of a hyperendemic community-associated clone rather than antibiotic
30
31 selection pressure alone contributed to this finding.
32
33
34
35
36
37

38 Molecular typing of the ESBL-PE isolates from our cohort showed that *E. coli* ST131
39
40 with CTX-M beta-lactamases (11/71, 15.5%) were the most common ESBL
41
42 mechanism, echoing the global dissemination of this hyperendemic clone, especially
43
44 in the community.[28] Similar reports showed 11.1% (32/287) in China[29] and 4.1%
45
46 (8/193) in Thailand[30] have been published. The reason for the rapid worldwide
47
48 expansion and long-term persistence of *E. coli* ST131 is thought to be due to
49
50 compensatory mutations within the core genome counterbalancing the fitness cost
51
52 associated with IncF plasmids, thus sustaining its spread even in the absence of direct
53
54 antibiotic selection pressure.[31] These *E. coli* ST131 are not just prevalent colonisers
55
56 but have also associated with invasive bloodstream infections in hospitalized patients
57
58
59
60

1
2
3 in Australia, New Zealand and Singapore.[32] It will be important to better
4
5 understand the evolutionary ecology and transmission dynamics of this emerging
6
7 clone.
8
9

10
11
12 This study also revealed widespread misconceptions about the utility of antibiotics for
13
14 viral infections, consistent with the findings of a global survey conducted by the
15
16 WHO in 2015.[33] We also found that, the public continues to place trust in their
17
18 primary care doctors and their recommendations. This dependence on physicians is in
19
20 contrast to doctors' perceptions of patient expectations for antibiotic
21
22 prescriptions.[34] This discordance has been previously described and is thought to be
23
24 due to the lack of empowerment of the patient and the erroneous attribution of patient
25
26 satisfaction to antibiotic prescription rather than a focus on better patient-doctor
27
28 communication.[35,36]
29
30
31
32
33

34
35 Engaging and educating both the prescribers and the public may reduce inappropriate
36
37 antibiotic use,[37,38] and has been identified as a key strategy by the WHO and the
38
39 UK to tackle AMR.[39,40] One of the most striking findings of this study is that
40
41 having both the knowledge that antibiotic courses should be completed and not
42
43 having left over antibiotics is independently associated with the carriage of ESBL-PE.
44
45 Though these relationships cannot be viewed as causal given the complexities in the
46
47 emergence and transmission of AMR, there is emerging evidence supporting short
48
49 course antibiotic therapies, even for severe infections such as bacteremia, given the
50
51 collateral damage that antibiotics have on host microbiome.[41] The current WHO
52
53 recommendation remains that full courses of antibiotics should be completed to
54
55 prevent the onset of resistance.[33] Similar messages are advocated in national
56
57
58
59
60

1
2
3 campaigns launched in Australia,[42] the United States[43] and Europe.[44] Given
4
5 that the minimum effective treatment durations have not been determined for many
6
7 infections and that a significant proportion of antibiotic prescriptions are
8
9 inappropriate, the emphasis on completing the course of antibiotics to prevent
10
11 resistance may have to be re-examined.
12
13
14
15
16

17 To our knowledge, this is the first study that explored antibiotic consumption
18
19 behavior with the acquisition of MDRO at a community level. This novel approach
20
21 has the potential to guide clinicians and policy makers in identifying directly
22
23 actionable interventions for the population. The main weakness of our study is that
24
25 the questionnaire data is self-reported and subjected to recall and interviewer biases.
26
27 We minimised these errors by designing specific questions that are carefully
28
29 constructed to maximize accuracy and completeness, and all interviewers were trained
30
31 to adhere to the question and answer format strictly. Further research using antibiotic
32
33 prescription databases can potentially overcome some of the intrinsic biases arising
34
35 from cross-sectional questionnaires.
36
37
38
39
40
41

42 **CONCLUSION**

43
44 There is a significant burden of asymptomatic ESBL-PE colonisation in Singapore,
45
46 especially with *E. coli* ST131 carrying CTX-M. This is correlated with KAP of
47
48 antibiotic use, especially with the practice of finishing full courses of antibiotics, and
49
50 prolonged residency in other parts of Asia. Innovative approaches to control AMR
51
52 that take into account transboundary transmission of resistance and clinical trials to
53
54 determine the appropriate duration of antimicrobial therapy will be critical to control
55
56
57
58
59
60

1
2
3 the emergence of these resistant clones which have contributed significantly to the
4
5 current global antibiotic resistance crisis.
6
7
8
9

10 **CONTRIBUTOR AND GUARANTOR INFORMATION**

11
12 YM, PAT, ARC, IS, PSPL, XLJK and KYMW conceptualised and designed the
13
14 study. IS, PSPL, XLJK and KYMW conducted the study and collected data. KKKK
15
16 performed microbiological testing. RTHO planned and conducted genomic
17
18 sequencing and interpreted the results. YM, ARC, IS, PSPL, XLJK and KYMW
19
20 performed data analysis. All participated in the writing of the script, and affirm that
21
22 the manuscript is an honest, accurate, and transparent account of the study being
23
24 reported; that no important aspects of the study have been omitted; and that any
25
26 discrepancies from the study as originally planned have been explained. YM and IS
27
28 accept full responsibility for the work and/or the conduct of the study, had access to
29
30 the data, and controlled the decision to publish. The corresponding author attests that
31
32 all listed authors meet authorship criteria and no others meeting the criteria have been
33
34 omitted.
35
36
37
38
39
40
41

42 **ACKNOWLEDGEMENT**

43
44 The authors thank the study team members, Ang Chen Xiang, Anne Goei Hui Yi,
45
46 Charmaine Loh Hui Yun, Cheong Shao Wei Dominic, Chew Shi Jie, Chong Yvette,
47
48 Choo Hui Min Charlotte, Choo Xin Yi, Daveraj Sivasegaran, Dean Krishen Sethi,
49
50 Joshua Tan Teck Chin, Keith Ching Wei Jie, Khoo Chun Yuet, Krystal Khoo Oon
51
52 Hui, Lai Jieru, Liew Yi Song Terence, Lim Li Liang Joshua, Lok Si Ying Andrea,
53
54 Lynette Sim Pei Shuen, Michelle Sim Yan Lin, Mok Charlene, Ong Yuxuan Daniel,
55
56 Ong Zheng Xuan, Quek Keng Liang, R Krishnapriya, Sophia Ng Shuen Yii, Tan Fang
57
58
59
60

1
2
3 Min Grace, Tan Jian Wei, Tan Pei Min Mabelleline, Tay Yiling Elaine, Tey Min Li,
4
5 Wu Yanlin, Zhou Lingyue, for their contributions in carrying out home visits,
6
7 interviews and sample collections.
8
9

10 11 12 **COPYRIGHT/LICENCE FOR PUBLICATION**

13
14 The Corresponding Author has the right to grant on behalf of all authors and does
15
16 grant on behalf of all authors, a worldwide licence to the Publishers and its licensees
17
18 in the perpetuity, in all forms, formats and media (whether known now or created in
19
20 the future), to i) publish, reproduce, distribute display and store the Contribution, ii)
21
22 translate the Contribution into other languages, create adaptations, reprints, include
23
24 within collections and create summaries, extracts and/or, abstracts of the
25
26 Contribution, iii) create any other derivative work(s) based on the Contribution, iv) to
27
28 exploit all subsidiary rights in the Contribution, v) the inclusion of electronic links
29
30 from the Contribution to third party material where-ever it may be located; and, vi)
31
32 licence any third party to do any or all of the above.
33
34
35
36
37
38
39

40 **FUNDING**

41
42 Data collection was supported from funding from the Infectious Diseases Research
43
44 Fund, National University of Singapore (NUS), and the Saw Swee Hock School of
45
46 Public Health (SSHSPH). RTHO received funding support from the SSHSPH, NUS.
47
48 ARC was supported by the Singapore Ministry of Health's National Medical
49
50 Research Council under the Centre Grant Programme - Singapore Population Health
51
52 Improvement Centre (NMRC/CG/C026/2017_NUHS).
53
54
55
56
57

58 **COMPETING INTERESTS DECLARATION**

1
2
3 All authors have completed the ICMJE uniform disclosure form at
4
5 www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any
6
7 organisations that might have an interest in the submitted work in the previous three
8
9 years; no other relationships or activities that could appear to have influenced the
10
11 submitted work.
12
13
14
15
16

17 DATA SHARING

18
19 The authors commit to making the relevant anonymised patient level data available on
20
21 reasonable request.
22
23
24
25

26 REFERENCES

- 27
28
29 1 Tacconelli E, Carrara E, Savoldi A, *et al.* Discovery, research, and
30
31 development of new antibiotics: the WHO priority list of antibiotic-resistant
32
33 bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27.
34
35 doi:10.1016/S1473-3099(17)30753-3
36
37
38 2 Ruppé É, Woerther P-L, Barbier F. Mechanisms of antimicrobial resistance in
39
40 Gram-negative bacilli. *Ann Intensive Care* 2015;**5**:61. doi:10.1186/s13613-015-
41
42 0061-0
43
44
45 3 Karanika S, Karantanos T, Arvanitis M, *et al.* Fecal Colonization With
46
47 Extended-spectrum Beta-lactamase–Producing *Enterobacteriaceae* and Risk
48
49 Factors Among Healthy Individuals: A Systematic Review and Metaanalysis.
50
51 *Clin Infect Dis* 2016;**63**:310–8. doi:10.1093/cid/ciw283
52
53
54 4 Reddy P, Malczynski M, Obias A, *et al.* Screening for Extended-Spectrum -
55
56 Lactamase-Producing *Enterobacteriaceae* among High-Risk Patients and Rates
57
58 of Subsequent Bacteremia. *Clin Infect Dis* 2007;**45**:846–52.
59
60

- 1
2
3 doi:10.1086/521260
4
5
6 5 Troché G, Toly L-M, Guibert M, *et al.* Detection and Treatment of Antibiotic-
7
8 Resistant Bacterial Carriage in a Surgical Intensive Care Unit: A 6-Year
9
10 Prospective Survey. *Infect Control Hosp Epidemiol* 2005;**26**:161–5.
11
12 doi:10.1086/502521
13
14
15 6 Coker RJ, Hunter BM, Rudge JW, *et al.* Emerging infectious diseases in
16
17 southeast Asia: regional challenges to control. *Lancet* 2011;**377**:599–609.
18
19 doi:10.1016/S0140-6736(10)62004-1
20
21
22 7 Zellweger RM, Carrique-Mas J, Limmathurotsakul D, *et al.* A current
23
24 perspective on antimicrobial resistance in Southeast Asia. *J Antimicrob*
25
26 *Chemother* 2017;**72**:2963–72. doi:10.1093/jac/dkx260
27
28
29 8 Singapore Department of Statistics. Singapore Residents by Planning
30
31 Area/Subzone, 2015. 2015.
32
33
34 9 Pan DST, Huang JH, Lee MHM, *et al.* Knowledge, attitudes and practices
35
36 towards antibiotic use in upper respiratory tract infections among patients
37
38 seeking primary health care in Singapore. *BMC Fam Pract* 2016;**17**:148.
39
40 doi:10.1186/s12875-016-0547-3
41
42
43 10 Scaioli G, Gualano MR, Gili R, *et al.* Antibiotic Use: A Cross-Sectional
44
45 Survey Assessing the Knowledge, Attitudes and Practices amongst Students of
46
47 a School of Medicine in Italy. *PLoS One* 2015;**10**:e0122476.
48
49 doi:10.1371/journal.pone.0122476
50
51
52 11 Awad AI, Aboud EA. Knowledge, attitude and practice towards antibiotic use
53
54 among the public in Kuwait. *PLoS One* 2015;**10**:e0117910.
55
56 doi:10.1371/journal.pone.0117910
57
58
59 12 Huang Y, Gu J, Zhang M, *et al.* Knowledge, attitude and practice of
60

- 1
2
3 antibiotics: a questionnaire study among 2500 Chinese students. *BMC Med*
4
5 *Educ* 2013;**13**:163. doi:10.1186/1472-6920-13-163
6
7
8 13 Lv B, Zhou Z, Xu G, *et al.* Knowledge, attitudes and practices concerning self-
9
10 medication with antibiotics among university students in western China. *Trop*
11
12 *Med Int Heal* 2014;**19**:769–79. doi:10.1111/tmi.12322
13
14
15 14 Teck KC, Ghazi HF, Bin Ahmad MI, *et al.* Knowledge, Attitude, and Practice
16
17 of Parents Regarding Antibiotic Usage in Treating Children’s Upper
18
19 Respiratory Tract Infection at Primary Health Clinic in Kuala Lumpur,
20
21 Malaysia. *Heal Serv Res Manag Epidemiol* 2016;**3**:233339281664372.
22
23 doi:10.1177/2333392816643720
24
25
26 15 Hall MA, Zheng B, Dugan E, *et al.* Measuring Patients’ Trust in their Primary
27
28 Care Providers. *Med Care Res Rev* 2002;**59**:293–318.
29
30 doi:10.1177/1077558702059003004
31
32
33 16 Performance Standards for Antimicrobial Susceptibility Testing An
34
35 informational supplement for global application developed through the Clinical
36
37 and Laboratory Standards Institute.
38
39 <http://ljzx.cqrmhospital.com/upfiles/201601/20160112155335884.pdf>
40
41 (accessed 12 Apr 2018).
42
43
44 17 Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: a new genome assembly
45
46 algorithm and its applications to single-cell sequencing. *J Comput Biol*
47
48 2012;**19**:455–77. doi:10.1089/cmb.2012.0021
49
50
51 18 Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification
52
53 using exact alignments. *Genome Biol* 2014;**15**:R46. doi:10.1186/gb-2014-15-3-
54
55 r46
56
57
58 19 Madden T. Chapter 16. The BLAST Sequence Analysis Tool.
59
60

- 1
2
3 [https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.p](https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf)
4 [df](https://www.ncbi.nlm.nih.gov/books/NBK21097/pdf/Bookshelf_NBK21097.pdf) (accessed 20 Apr 2018).
5
6
7
8 20 Inouye M, Dashnow H, Raven L-A, *et al*. SRST2: Rapid genomic surveillance
9 for public health and hospital microbiology labs. *Genome Med* 2014;**6**:90.
10 doi:10.1186/s13073-014-0090-6
11
12
13
14
15 21 R Core Team. A language and environment for statistical computing. 2017.
16
17 22 Young BE, Lye DC, Krishnan P, *et al*. A prospective observational study of the
18 prevalence and risk factors for colonization by antibiotic resistant bacteria in
19 patients at admission to hospital in Singapore. *BMC Infect Dis* 2014;**14**:298.
20 doi:10.1186/1471-2334-14-298
21
22
23
24
25
26 23 McNulty CAM, Lecky DM, Xu-McCrae L, *et al*. CTX-M ESBL-producing
27 Enterobacteriaceae: estimated prevalence in adults in England in 2014. *J*
28 *Antimicrob Chemother* Published Online First: 5 March 2018.
29 doi:10.1093/jac/dky007
30
31
32
33
34
35 24 Tängdén T, Cars O, Melhus A, *et al*. Foreign travel is a major risk factor for
36 colonization with Escherichia coli producing CTX-M-type extended-spectrum
37 beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob*
38 *Agents Chemother* 2010;**54**:3564–8. doi:10.1128/AAC.00220-10
39
40
41
42
43
44 25 Woerther P-L, Andremont A, Kantele A. Travel-acquired ESBL-producing
45 Enterobacteriaceae: impact of colonization at individual and community level.
46 *J Travel Med* 2017;**24**:S29–34. doi:10.1093/jtm/taw101
47
48
49
50
51 26 Augustine MR, Testerman TL, Justo JA, *et al*. Clinical Risk Score for
52 Prediction of Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae
53 in Bloodstream Isolates. *Infect Control Hosp Epidemiol* 2017;**38**:266–72.
54
55
56
57
58
59
60

- 1
2
3 27 Tumbarello M, Treccarichi EM, Bassetti M, *et al.* Identifying Patients
4
5 Harboring Extended-Spectrum- β -Lactamase-Producing Enterobacteriaceae on
6
7 Hospital Admission: Derivation and Validation of a Scoring System.
8
9
10 *Antimicrob Agents Chemother* 2011;**55**:3485–90. doi:10.1128/AAC.00009-11
11
12 28 Dautzenberg MJD, Haverkate MR, Bonten MJM, *et al.* Epidemic potential of
13
14 Escherichia coli ST131 and Klebsiella pneumoniae ST258: a systematic review
15
16 and meta-analysis. *BMJ Open* 2016;**6**:e009971. doi:10.1136/bmjopen-2015-
17
18 009971
19
20
21 29 Zhong Y-M, Liu W-E, Liang X-H, *et al.* Emergence and spread of O16-ST131
22
23 and O25b-ST131 clones among faecal CTX-M-producing Escherichia coli in
24
25 healthy individuals in Hunan Province, China. *J Antimicrob Chemother*
26
27 2015;**70**:2223–7. doi:10.1093/jac/dkv114
28
29
30 30 Niumsup PR, Tansawai U, Na-udom A, *et al.* Prevalence and risk factors for
31
32 intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai
33
34 community. *Eur J Clin Microbiol Infect Dis* 2018;**37**:69–75.
35
36 doi:10.1007/s10096-017-3102-9
37
38
39 31 Pitout JDD, DeVinney R. Escherichia coli ST131: a multidrug-resistant clone
40
41 primed for global domination. *F1000Research* 2017;**6**.
42
43 doi:10.12688/f1000research.10609.1
44
45
46 32 Harris PNA, Ben Zakour NL, Roberts LW, *et al.* Whole genome analysis of
47
48 cephalosporin-resistant Escherichia coli from bloodstream infections in
49
50 Australia, New Zealand and Singapore: high prevalence of CMY-2 producers
51
52 and ST131 carrying blaCTX-M-15 and blaCTX-M-27. *J Antimicrob*
53
54 *Chemother* 2018;**73**:634–42. doi:10.1093/jac/dkx466
55
56
57 33 Antibiotic resistance: Multi-country public awareness survey. World Heal.
58
59
60

- 1
2
3 Organ.
4
5 2016.<http://www.who.int/drugresistance/documents/baselinesurveynov2015/en>
6
7 / (accessed 25 Mar 2018).
8
9
- 10 34 Lee T-H, Wong JG, Lye DC, *et al.* Medical and psychosocial factors associated
11 with antibiotic prescribing in primary care: survey questionnaire and factor
12 analysis. *Br J Gen Pract* 2017;**67**:e168–77. doi:10.3399/bjgp17X688885
13
14
- 15 35 Welschen I, Kuyvenhoven M, Hoes A, *et al.* Antibiotics for acute respiratory
16 tract symptoms: patients' expectations, GPs' management and patient
17 satisfaction. *Fam Pract*; **21**. doi:10.1093/fampra/cmh303
18
19
- 20 36 Davey P, Pagliari C, Hayes A. The patient's role in the spread and control of
21 bacterial resistance to antibiotics. *Clin Microbiol Infect* 2002;**8**:43–68.
22 doi:10.1046/J.1469-0691.8.S.2.6.X
23
24
- 25 37 McNulty CAM, Cookson BD, Lewis MAO. Education of healthcare
26 professionals and the public. *J Antimicrob Chemother* 2012;**67**:i11–8.
27 doi:10.1093/jac/dks199
28
29
- 30 38 Little P, Stuart B, Francis N, *et al.* Effects of internet-based training on
31 antibiotic prescribing rates for acute respiratory-tract infections: a
32 multinational, cluster, randomised, factorial, controlled trial. *Lancet*
33 2013;**382**:1175–82. doi:10.1016/S0140-6736(13)60994-0
34
35
- 36 39 UK Five Year Antimicrobial Resistance Strategy 2013 to 2018.
37 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/att](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/244058/20130902_UK_5_year_AMR_strategy.pdf)
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 40 WHO Global Strategy for Containment of Antimicrobial Resistance WHO
Global Strategy for Containment of Antimicrobial Resistance. World Heal.

- 1
2
3 Organ. http://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf
4
5 (accessed 5 Apr 2018).
6
7
8 41 Llewelyn MJ, Fitzpatrick JM, Darwin E, *et al.* The antibiotic course has had its
9
10 day. *BMJ* 2017;;j3418. doi:10.1136/bmj.j3418
11
12 42 Antibiotics and antibiotic resistance. NPS MedicineWise.
13
14 2018.[https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-](https://www.nps.org.au/medical-info/consumer-info/antibiotic-resistance-the-facts)
15
16 the-facts (accessed 10 Apr 2018).
17
18
19 43 Consumer Updates - Combating Antibiotic Resistance.
20
21 2018.<https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm092810.htm>
22
23 (accessed 10 Apr 2018).
24
25
26 44 Factsheet for the general public - Antimicrobial resistance.
27
28 [https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-](https://ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-public)
29
30 public (accessed 10 Apr 2018).
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

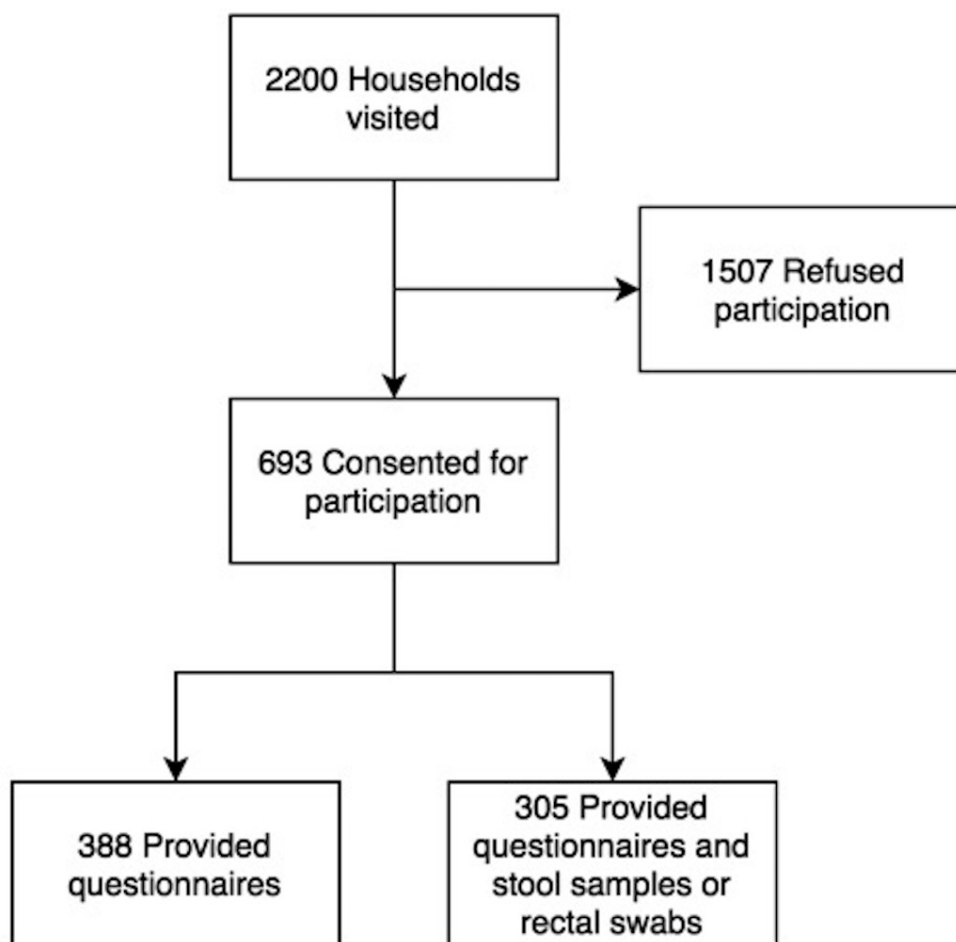


Figure S1. Participant recruitment flowchart

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. Occupation:

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

1
2
3 5.3 Have you travelled to the following places within the past 6 months? – Yes or

4
5 No

6
7
8 5.3.1 If yes, which of the following places have you been to? (You may select more
9
10 than 1 option) - Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam,
11
12 Cambodia etc) and/ or South Asia (India, Bangladesh, Sri Lanka) and/ or East
13
14 Asia (China, Korea, Japan) and/ or Europe and/ or South America and/ or
15
16 North America and/ or Middle East or Others:

17
18
19 5.4 Have you lived anywhere else for more than 1 year? – Yes or No

20
21
22 6 If yes, did you live in the following areas? (You may select more than 1 option) -
23
24 Southeast Asia (Malaysia, Thailand, Indonesia, Vietnam, Cambodia etc) and/ or
25
26 South Asia (India, Bangladesh, Sri Lanka) and/ or East Asia (China, Korea,
27
28 Japan) and/ or Europe and/ or South America and/ or North America and/ or
29
30 Middle East or Others:

31
32
33 7 Medical History

34
35 6.1 Do you have any of the following? (You can choose more than one of the
36
37 following) - Diabetes Mellitus and/ or Medications (Chemotherapy, Steroids,
38
39 Immunosuppressants etc) and/ or Other medical conditions or None of the above

40
41
42 6.2 When was your last hospitalisation? - Never been hospitalised before or
43
44 Hospitalised before

45
46
47 6.2.1 If yes, was this hospitalisation within the past 1 year? – Yes or No

48
49 6.2.2 How long was your stay? Duration:

50
51
52 6.3 Have you used antibiotics before? - Have never used antibiotics before or Used
53
54 antibiotics before

55
56 6.3.1 If yes, when was the last time you started on antibiotics? - Within the last 6
57
58
59
60

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

1
2
3 or Keep it for future use or Pour it down a sink or toilet bowl or Disposal in the
4 rubbish bin or Others:
5
6
7

8
9 **3. Assessing Alternative Antibiotic Practices**

10
11 3.1 Have you ever shared antibiotics with someone else? – Yes or No

12
13
14 3.2 Have you ever taken leftover antibiotics from a previous course of illness? – Yes
15 or No
16
17
18
19

20
21
22 **Section 3: Attitude Assessment**

23
24
25 **1. Attitudes Towards Healthcare Provider Prescription**

26
27 1.1 Sometimes my doctor prioritises what is beneficial for him over my medical
28 needs. – Strongly agree or Agree or Neutral or Disagree or Strongly Disagree
29

30
31 1.2 My doctor's medical skills are not as good as they should be. – Strongly agree or
32 Agree or Neutral or Disagree or Strongly Disagree
33
34

35
36 1.3 My doctor is always honest when telling me about all the available treatments for
37 my condition. – Strongly agree or Agree or Neutral or Disagree or Strongly
38 Disagree
39
40
41
42

43
44 1.4 I have no worries about putting my life in my doctor's hands. – Strongly agree or
45 Agree or Neutral or Disagree or Strongly Disagree
46
47

48
49 **2. Attitudes Towards Potential Educational Interventions**

50
51 2.1 Which of the following sources of medical information do you trust most? -

52
53 Healthcare Professionals' Advice (Doctors, nurses, clinical assistants, therapists)
54 or Family and Friends or Online Medical Sources or Television Programmes and
55 Advertisements or Radio Programmes and Advertisements
56
57
58
59
60

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

1
2
3 becomes weaker or Your body immunity becomes stronger
4

5 4.3 Antimicrobial resistance is not present in Singapore yet. – Yes or No or I am
6

7
8 unsure
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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%)

1 2 3 4 5 6 7 8 9	2.1.8	What causes antimicrobial resistance? (Open ended)	Inappropriate use of antibiotics	121 (17.5%)
			Wrong or unsure	572 (82.5%)
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34	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%)
35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	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)

For peer review only

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

	<i>E coli</i>			p-value
	N=71 (%)			
	ST131 N=11 (%)	Non ST131 N=60 (%)		
Number of resistant genes (mean \pm sd)		1.2 \pm 0.4	1.9 \pm 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)

For peer review only

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	4-5

		<i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	NA
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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.

1
2
3
4 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE
5 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
6 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

For peer review only