Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* in Retail Chicken Meat in Singapore

Dear Editor,

Extended-spectrum beta-lactamases (ESBLs) are rapidly expanding groups of enzymes that can hydrolyse the majority of beta-lactam antibiotics with the exception of carbapenems, and are inhibited by clavulanic acid.[1] They are commonly found on plasmids, which are extra-chromosomal deoxyribonucleic acid (DNA) that can transfer between bacteria. ESBL-producing *Enterobacteriaeceae* (ESBL-E) frequently cause infections in Singapore hospitals, and have increased rapidly since the 1990s to approximately 20% and 35% of all *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) clinical isolates respectively.[2]

ESBL-E carriage and infection rates in the community setting have increased globally since the 1990s.[3] In Singapore, 6.3% of emergency department attendees in 2006 with no previous healthcare contact were ESBL-E carriers—unfortunately, there has been no follow-up study.[4] It is postulated that the rise in community-associated ESBL-E carriage and infection rates is due to cross-species transmission from food-producing animals, particularly poultry.[3,5,6] Numerous studies have shown high rates of ESBL-E in chickens around the world.[3,6-8] Citing the Agri-Food and Veterinary Authority of Singapore (AVA), a 2013 news report stated that Singapore imported approximately 500 tons of chicken a day, of which 78.8% came from Brazil (205 tons) and Malaysia (189 tons).[9]

We performed a cross-sectional survey on 26 chicken breast samples over a period of 4 months (May 2015 to August 2015) in an attempt to determine the prevalence of ESBL-E in chicken meat in Singapore, and to determine if there was a difference in ESBL-E carriage between raw and commercially cooked chicken.

Raw chilled and frozen meat were obtained from various wet markets, supermarkets, and online shops (n = 19), whereas cooked samples were obtained from fast food restaurants and hawker centres (n = 7). Samples were labelled as "antibiotic-free" if this was indicated on the packaging. The country of origin was obtained from the packaging label, or from the seller in the case of wet market samples.

Samples were processed under aseptic conditions within 4 hours of collection. Approximately 25 grams of breast meat was macerated in a stomacher with 225 mL buffered peptone water at 260 rpm for 1 minute, and incubated for 18 to 24 hours at 35°C. Subsequently, the homogenate was diluted 1:100 in peptone water and 10 μL of the resulting mix was plated on selective ChromID ESBL agar (Biomerieux, France). After incubation overnight, several colonies with distinct morphologic appearances per plate were randomly selected for further testing and identified using MALDI-TOF (Bruker Daltonics, Germany), with confirmation of *E. coli* identification via standard microbiology testing.[10]

Phenotypic confirmation of ESBL production was made according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] ESBL-E were screened for the presence of genes expressing the CTX-M subclass of beta-lactamase enzymes (CTX-M) using previously described multiplex polymerase chain reaction (PCR) methods.[12,13] Confirmation of the CTX-M group was performed via Sanger sequencing (performed commercially by AITbiotech, Singapore), with consensus sequences compared to existing sequences within the National Center for Biotechnology Information (NCBI) databases.

The majority of the raw samples were fresh chilled chicken from Malaysia (n = 11, 57.9%), with the rest being frozen chicken from France (21.1%), Brazil (10.5%), and the United States of America (USA) (10.5%). Seven samples of "antibiotic-free" chicken originated from Malaysia (n = 3, 42.9%), France (28.6%) and USA (28.6%). The countries of origin of the cooked samples were presumably from either Malaysia or Brazil.

The ChromID ESBL screening plates detected ESBL-E in 15 (78.9%) raw samples and none of the cooked samples. Eleven of 12 (91.7%) samples from conventionally raised chickens harboured ESBL-E, compared to 4 of 7 (57.1%) "antibiotic-free" samples. Fifty-six of the colonies tested were confirmed to be ESBL-E phenotypically (Table 1). The majority was *E. coli* (82.1%), followed by *Proteus mirabilis* (10.7%) and *K. pneumoniae* (7.1%).

Multiplex PCR revealed that 54 ESBL-E (96.4%) harboured CTX-M genes. CTX-M-1 group genes were found in the most isolates (n = 28, 51.9%), followed by the CTX-M-9 group genes (n = 17, 31.5%), CTX-M-2 group genes (n = 9, 16.7%), and CTX-M-8 group genes

Table 1. Distribution of CTX-M Genes and *Enterobacteriaceae* Isolates from 15 Chicken Samples According to Type of Chicken and Country of Origin

Country of Origin	Type of Chicken	Poultry Farming	*Enterobacteriaceae* (Number of Isolates)	CTX-M Group (Number of Isolates)
Malaysia	Black (ayam cemani[†])	Conventional	*Escherichia coli* (2)	1 (2)
Malaysia	Ordinary	Conventional	*E. coli* (2)	1 (1)
				9 (1)
Malaysia	Ordinary	Conventional	*E. coli* (2)	9 (2)
Malaysia	Ordinary	Conventional	*E. coli* (4)	9 (3)
				2 and 9 (1)
Malaysia	Ordinary (ayam kampong[‡])	Conventional	*E. coli* (2)	1 (1)
				9 (1)
Malaysia (France)[*]	Yellow chicken	Conventional	*E. coli* (2)	1 (2)
Malaysia	Ordinary	Antibiotic-free (probiotic)	*E. coli* (3)	1 (3)
Malaysia	Ordinary	Antibiotic-free (probiotic)	*E. coli* (3)	2 and 9 (2)
				9 (1)
Malaysia	Ordinary	Antibiotic-free (probiotic)	*E. coli* (1)	1 (1)
			Proteus mirabilis (1)	9 (1)
			Klebsiella pneumoniae (2)	CTX-M negative
Malaysia	Ordinary	Antibiotic-free (probiotic)	*E. coli* (2)	1 (2)
			P. mirabilis (5)	9 (5)
			K. pneumoniae (2)	1 (2)
Brazil	Ordinary	Conventional	*E. coli* (5)	2 (2)
				8 (3)
Brazil	Ordinary	Conventional	*E. coli* (4)	2 (4)
France	Ordinary	Conventional	*E. coli* (4)	1 (4)
France	Yellow chicken	Conventional	*E. coli* (5)	1 (5)
France	Yellow chicken	Antibiotic-free	*E. coli* (5)	1 (5)

[*]A French chicken breed but raised on Malaysian farms.
[†]Black chicken.
[‡]Chickens raised using traditional free range production techniques.

(n = 3, 5.5%). Three (5.6%) isolates had both CTX-M-2 and CTX-M-9 group genes, while the CTX-M genes of 2 isolates could not be grouped.

Our study, even with its limited sample size, showed very high percentages of ESBL-E carriage in retail chicken in Singapore, comparable if not higher than most other reports from around the world.[5,7-9] Even in chicken that were ostensibly raised antibiotic-free, many samples were found to be positive for ESBL-E. The vast majority of chicken samples from Malaysia (90.9%) and Brazil (100%) tested positive for ESBL-E.

The vast majority of ESBL-E carried CTX-M genes, which is unsurprising given the success of this group of ESBL genes in *Enterobacteriaceae* of both animal and human origin.[3,5,6] It is likely that at least 2 isolates carried the older TEM and SHV-ESBL genes, but these were not tested. It is noteworthy that CTX-M-positive *Enterobacteriaceae* were first noted in human clinical isolates in Singapore in the 1990s,[2] and gradually became the predominant ESBL in Singapore hospitals by the mid-2000s,[2,14] with an increasing number of sporadic community-associated ESBL-E carrying CTX-M genes seen in the past several years.[14] A small but significant percentage of the community had already been found to be colonised by ESBL-E in 2006, with the majority (74.4%) of the isolates testing positive for CTX-M genes, primarily CTX-M-1 and CTX-M-9 groups.[4]

One silver lining of this study is that none of the cooked samples tested positive for ESBL-E, suggesting that thorough cooking may minimise the transmission of ESBL-E. However, given the presence of community human carriage of ESBL-E with CTX-M groups similar to those found in ESBL-E from chicken,[4] it is plausible that cross-transmission has occurred and continues to occur. How cross-transmission takes place locally is speculative

at best, but perhaps transpires during food preparation, or via ingestion of large amounts of less well cooked chicken. Food sources other than meat have also been found to be positive for ESBL-E, including vegetables,[15] and this may contribute to the overall colonising pressure of ESBL-E on humans in Singapore.

Our study is primarily limited by the small sample size, although the results are striking enough that a larger sample size would not necessarily yield more significant results. Further molecular work could be performed to determine whether the ESBL-E from the chicken samples corresponded to human pathogenic clones of *Enterobacteriaceae*. However, such work has already been performed elsewhere,[6] and in any case, the issue is not primarily whether the bacteria from chicken are pathogenic to humans, but that these antibiotic resistance determinants can easily be transferred between animal and human (pathogenic) bacterial strains. Because our methodology specifically required bacterial cultures, it may not identify ESBL-E at low concentrations or other ESBL-producing bacteria that are unculturable using these methods, and alternative direct PCR-based techniques may be complementary.

What can be done concretely with regard to the overall issue of antimicrobial resistance in food products is less clear. Singapore imports the majority of its food,[10] and has limited ability to influence agricultural producers with regard to antibiotic and farming practices. Perhaps heightened awareness of the issue of antimicrobial resistance, framed as a food safety or health issue, may result in local consumers exerting greater economic selection pressure in terms of antibiotic-free (or better yet, antibiotic resistance-free) food products.

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Eugene JZ Lim*, [1], Si Xian Ho*, [1], Delphine YH Cao, [2 BSc], Quek Choon Lau, [1 PhD], Tse Hsien Koh, [2 PhD], Li Yang Hsu, [3,4 MPH]

[1]Life Sciences and Chemical Technology, Ngee Ann Polytechnic, Singapore
[2]Department of Pathology, Singapore General Hospital, Singapore
[3]Mount Elizabeth Novena Hospital, Singapore
[4]Saw Swee Hock School of Public Health, National University Health System, Singapore

Address for Correspondence: Dr Hsu Li Yang, Saw Swee Hock School of Public Health, National University Health System, Tahir Foundation Building, 12 Science Drive, Singapore 117549.
Email: liyang_hsu@yahoo.com
*These authors contributed equally to the work.