Research Paper

Foodborne *Klebsiella pneumoniae*: Virulence Potential, Antibiotic Resistance, and Risks to Food Safety

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ABSTRACT

Gastrointestinal carriage of *Klebsiella pneumoniae* is a predisposing factor for liver abscess in several Asian countries. To determine whether hypervirulent *K. pneumoniae* in the gut may be transmitted through food, we screened a range of raw and ready-to-eat retail food by culture and recovered *K. pneumoniae* in 21% (147 of 698) of samples tested. Based on PCR, no *K. pneumoniae* isolates carried the *rmpA* gene linked to community-acquired pyogenic liver abscess, providing no evidence of a link between food and liver disease. However, phenotypic resistance to multiple antibiotic classes was seen through disk diffusion tests, and carriage of genetic elements (*wcaG* and capsule types K1, K2, and K54) associated with increased virulence (8%, 11 of 147) was observed by PCR. Multidrug-resistant isolates were from raw vegetables, chicken or pork liver, and a ready-to-eat poultry dish; one multidrug-resistant *K. pneumoniae* isolate from raw bean sprouts was resistant to a third-generation cephalosporin (ceftriaxone). Although *K. pneumoniae* may be present in food without causing harm, we found isolates belonging to the K1 capsular serotype coexisting with the *wcaG* gene, one also conferring multidrug resistance. *K. pneumoniae* that carry antibiotic resistance genes, regardless of pathogenicity, may increase the available genetic pool of resistance along the food chain. Hygienic food handling practices are necessary to lower risks of acquiring *K. pneumoniae* and other opportunistic pathogens.

HIGHLIGHTS

- Multidrug-resistant and potentially virulent *K. pneumoniae* was found in food.
- Food samples did not carry hypervirulent *K. pneumoniae* linked to liver abscess.
- Foodborne *K. pneumoniae* can be a potential reservoir of antibiotic resistance genes.
- Good food and personal hygiene are needed to help curb antimicrobial resistance.

Key words: Antibiotic resistance; Food; K1 *K. pneumoniae*; *Klebsiella pneumoniae*; *rmpA*

*Klebsiella pneumoniae* is an opportunistic bacterium found in various microbiological niches such as soil; the skin, intestines, and feces of mammals; and food. *K. pneumoniae* has been documented to cause bacteremia, pneumonia, and urinary tract infection (26, 42); the gastrointestinal carriage of *K. pneumoniae* has been said to be a predisposing factor for liver abscess (18), and hypervirulent *K. pneumoniae* strains have emerged as a predominant cause of pyogenic liver abscess in Asia (32). Studies have reported that most pathogens in liver abscess are susceptible to broad-spectrum antibiotics such as fluoroquinolones and third- and fourth-generation cephalosporins (18, 39, 43), but over the years, antibiotic resistance in *K. pneumoniae* has been increasingly observed in both nosocomial and community settings. Reports of *K. pneumoniae* having developed an acquired resistance to last-line antibiotics (i.e., carbapenems) (3, 35, 36) have been most concerning. The interplay between mechanisms of antibiotic resistance and virulence in *K. pneumoniae* is not well established. It is generally accepted that antibiotic resistance comes with a fitness cost and decreased virulence, but recent studies have suggested otherwise—the development of antibiotic resistance in *K. pneumoniae* has been reported to augment virulence (27), and increased virulence is said to naturally evolve in response to, or potentially be shared among, bacteria, leading to acquired resistance (41).
Although *K. pneumoniae* is more commonly associated with nosocomial infections, food has also been reported to be a possible transmission vector (5). *K. pneumoniae* has been isolated from raw meat (9, 21, 28, 48), raw vegetables (16, 38), fruit juice (19), and ready-to-eat (RTE) food (22, 40). Several studies on *K. pneumoniae* in food have also reported its worrying resistance to antibiotics, on several occasions citing foodborne *K. pneumoniae* as resistant to three or more antibiotic classes (multidrug resistance) (21, 22, 38). The presence of antimicrobial-resistant and multidrug-resistant (MDR) *K. pneumoniae* strains in the food chain and its potential contribution to the resistome, particularly to the resistance of clinically relevant bacteria, should not be taken lightly. The World Health Organization (WHO), in its global action plan against antimicrobial resistance, has identified that food is one of the potential vehicles for transmission of antimicrobial-resistant bacteria to humans and that human consumption of food carrying antimicrobial-resistant bacteria has led to the acquisition of antibiotic-resistant infections (47). The WHO has classified carbapenem-resistant and third-generation cephalosporin–resistant Enterobacteriaceae (including *K. pneumoniae*) as critical priority pathogens in its list of antibiotic-resistant bacteria needing new treatments.

Incidents of food contamination caused by agents other than the more frequently reported pathogens such as *Salmonella, Escherichia coli*, and *Campylobacter* are not unheard of (6, 25, 44). In Singapore, more than 90% of the local food supply is imported, and it is thus prudent to exercise vigilance to common and unexpected contaminants in food, such as *K. pneumoniae* and other bacteria known to harbor antibiotic resistance. In Singapore, information such as prevalence rates in retail food, antibiotic resistance profiles, and virulence characteristics that are needed to assess risks of foodborne *K. pneumoniae* to public health are limited. Local incidence rates of foodborne *K. pneumoniae* causing illness are also unavailable. With these knowledge gaps in mind, a three-part baseline study on *K. pneumoniae* in retail food was initiated as a collaboration between the National Environment Agency and the Singapore General Hospital. This study aimed to determine the positivity rate, virulence potential, and antibiotic resistance profiles of foodborne *K. pneumoniae*. The data gathered will contribute to our understanding of the potential risk of *K. pneumoniae* to retail food hygiene, food safety, and public health in Singapore.

**MATERIALS AND METHODS**

**General workflow.** A study was carried out to better understand the prevalence of *K. pneumoniae* in retail raw and cooked food. This study involved testing raw and RTE food available at retail for *K. pneumoniae*: molecular screening of isolated *K. pneumoniae* for virulence determinants and then testing of isolates for resistance against a range of antibiotics. The general workflow of the study is provided in Figure 1.

**Sampling of raw and RTE food.** A total of 698 food samples were collected for microbiological analysis from 2014 to 2017. Of these, 146 samples were raw food items from wet markets and supermarkets and 552 samples were RTE food dishes collected from hawker centers in Singapore. Upon purchase, samples were kept on ice and immediately brought to the laboratory for microbiological testing. The selection and collection of RTE food samples was part of a larger, separate
surveillance program that determines the microbiological quality of RTE food at Singapore hawker centers. The food items are listed in Table 1.

Detection of *K. pneumoniae* in food samples. For each food sample, 10 g was suspended in 90 mL of universal preenrichment broth (Acumedia, Lansing, MI) and homogenized for 30 s at 230 rpm in a Seward Stomacher 400 Circulator. The suspension was then incubated at 37°C for 24 h. After incubation, a 10-μL loopful of the suspension was streaked onto MacConkey agar (Oxoid, Basingstoke, UK) and incubated again at 37°C for 24 h. Colonies exhibiting pink mucoid morphology typical of *K. pneumoniae* were picked and regrown on tryptic soy agar (TSA; Oxoid) for 18 ± 2 h to obtain fresh cultures needed for further confirmation by PCR and API 20E (bioMérieux, Marcy l’Etoile, France).

**TABLE 1. Positivity, virulence, and multidrug resistance of *K. pneumoniae* in raw and ready-to-eat food samples**

<table>
<thead>
<tr>
<th>Food type</th>
<th><em>K. pneumoniae</em> positive samples, % (n/total n)<em>a</em></th>
<th><em>K. pneumoniae</em> isolate with virulence factor, % (n/total n)</th>
<th>Virulence factors (n)</th>
<th>Multidrug resistance (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean sprouts</td>
<td>80 (8/10)</td>
<td>0 (0/8)</td>
<td>—</td>
<td>AMP-C-CRO (1)</td>
</tr>
<tr>
<td>Spring onions</td>
<td>80 (8/10)</td>
<td>0 (0/8)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Chilies</td>
<td>75 (9/12)</td>
<td>11 (1/9)</td>
<td>K1, wcaG (1)<em>c</em></td>
<td>AMP-TE-AK (1)<em>c</em>, AMP-TE-C-SXT (1)</td>
</tr>
<tr>
<td>Parsley</td>
<td>70 (7/10)</td>
<td>0 (0/7)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>53 (8/15)</td>
<td>25 (2/8)</td>
<td>K1, wcaG (1), wcaG (1)</td>
<td></td>
</tr>
<tr>
<td>Cucumbers</td>
<td>42 (5/12)</td>
<td>0 (0/5)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>30 (3/10)</td>
<td>33 (1/3)</td>
<td>K1, wcaG (1)</td>
<td></td>
</tr>
<tr>
<td>Ginger</td>
<td>29 (2/7)</td>
<td>0 (0/2)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Chicken liver</td>
<td>27 (8/30)</td>
<td>0 (0/8)</td>
<td>—</td>
<td>AMP-C-CIP (1), AMP-TE-CIP (2), AMP-TE-CIP-SXT (1), AMP-TE-CIP-NA-SXT (1)</td>
</tr>
<tr>
<td><strong>Total, raw food</strong></td>
<td>45 (66/146)</td>
<td>8 (5/66)</td>
<td>K2 (1)</td>
<td>AMP-TE-C-CIP-SXT (1)</td>
</tr>
<tr>
<td><strong>Ready-to-eat (RTE)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popiah</td>
<td>33 (3/9)</td>
<td>0 (0/3)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Poultry dishes</td>
<td>27 (14/52)</td>
<td>14 (2/14)</td>
<td>wcaG (2)</td>
<td>AMP-AMC-TE-C-SXT (1)</td>
</tr>
<tr>
<td>Mixed vegetable rice dishes</td>
<td>25 (13/52)</td>
<td>0 (0/13)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Porridge</td>
<td>25 (3/12)</td>
<td>33 (1/3)</td>
<td>wcaG (1)</td>
<td>—</td>
</tr>
<tr>
<td>Fruit slices/ juice</td>
<td>19 (9/48)</td>
<td>0 (0/9)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bean dishes</td>
<td>14 (2/14)</td>
<td>0 (0/2)</td>
<td>—</td>
<td>NA<em>d</em></td>
</tr>
<tr>
<td>Noodle dishes</td>
<td>14 (19/138)</td>
<td>5 (1/19)</td>
<td>K54, wcaG (1)</td>
<td>—e*</td>
</tr>
<tr>
<td>Bean curd</td>
<td>11 (1/9)</td>
<td>0 (0/1)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Rice dishes</td>
<td>11 (1/9)</td>
<td>0 (0/1)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Seafood dishes</td>
<td>11 (3/27)</td>
<td>0 (0/3)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Yong tau foo</td>
<td>10 (1/10)</td>
<td>0 (0/1)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Bread/cakes</td>
<td>9 (4/43)</td>
<td>0 (0/4)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Beverages</td>
<td>7 (5/72)</td>
<td>20 (1/5)</td>
<td>K2 (1)</td>
<td>—e*</td>
</tr>
<tr>
<td>Pork dishes</td>
<td>6 (2/36)</td>
<td>50 (1/2)</td>
<td>wcaG (1)</td>
<td>—e*</td>
</tr>
<tr>
<td>Carrot cake</td>
<td>0 (0/13)</td>
<td>0 (0/0)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>Others</td>
<td>13 (1/8)</td>
<td>0 (0/1)</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Total, RTE food</strong></td>
<td>15 (81/552)</td>
<td>7 (6/81)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Total, all food types</strong></td>
<td>21 (147/698)</td>
<td>8 (11/147)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* As confirmed by PCR.

b —, virulence factor or multidrug resistance was not detected.

c Same *K. pneumoniae* isolate.

d NA, not applicable; *K. pneumoniae* isolates not tested for antibiotic resistance.

e For these food types, only potentially virulent *K. pneumoniae* was tested for antibiotic resistance.

**Confirmation of *K. pneumoniae* by PCR.** Nucleic acids were extracted from *K. pneumoniae* isolates by an in-house boiling method. Suspected *K. pneumoniae* colonies were picked from TSA and inoculated into individual vials containing 200 μL of Tris buffer (1× tetracycline [TE] buffer, pH 8.0; Amresco, Solon, OH). Each vial was heated at 95°C for 10 min; vials were then cooled on ice for another 10 min and centrifuged at 8,000 × g for 20 min. To perform analysis by PCR, 100 μL (minimum concentration of ~50 ng/μL) of DNA were kept, using the primers and conditions described by Liu et al. (31) and Turton et al. (45), provided in Table 2. The amplified products were visualized using a Qiaxcel DNA Screening kit (Qiagen, Hilden, Germany).

**Biochemical confirmation of *K. pneumoniae* by API 20E.** Isolates confirmed as *K. pneumoniae* by PCR were further analyzed by API 20E to verify the identity of colonies to be used for
antibiotic resistance testing. Suspected K. pneumoniae colonies were plated on TSA and incubated for 18 ± 2 h. Colonies were transferred into 5% saline to reach a ~0.5 McFarland standard turbidity. The suspension of bacteria and saline were then inoculated into each well of the color API 20E test strip according to the manufacturer’s instructions; test strips were then incubated at 37°C overnight. Changes were interpreted using the API 20E color guide; corresponding numerical codes were entered into the API Web online resource (www.apiweb.com) to determine the identity of the organism. Confirmed K. pneumoniae isolates were stored in brain heart infusion broth (Neogen, Lansing, MI) with 15% glycerol at −80°C.

Screening for virulence determinants in foodborne K. pneumoniae isolates. When isolates were confirmed by PCR to be K. pneumoniae, DNA were re-extracted using the DNeasy Blood & Tissue Kit (Qiagen) and sent to Singapore General Hospital Pathology Laboratory to test for virulence determinants (rmpA, wcaG, and capsular types K1, K2, K54, K57, K5, and K20) according to Turton et al. (45).

Antibiotic resistance profiling of foodborne K. pneumoniae isolates. The following K. pneumoniae isolates were selected for antibiotic resistance profiling: isolates from raw food regardless of whether virulence factors were detected (n = 66) and isolates from RTE food that had at least one virulence factor or had a K. pneumoniae positivity rate of at least 15% (n = 42). Out of these, however, 11 isolates could not be resuscitated from frozen glycerol stocks, so in total, 97 K. pneumoniae isolates were analyzed for susceptibility to antibiotics.

Susceptibility was tested toward 11 antibiotics (micrograms) belonging to the following eight classes: penicillin (amoxicillin–clavulanic acid, AMC 30; ampicillin, AMP 10), cephalosporin (third-generation cephraxone, CRO 30), tetracycline (TE 30), chloramphenicol (C 30), aminoglycoside (amikacin, AK 30; gentamicin, CN 10), sulfanamide (sulfamethoxazole-trimetoprim, SXT 25), quinolone (nalidixic acid, NA 30), and fluoroquinolone (ciprofloxacin, CIP 5; norfloxacin, NOR 10) based on the Kirby-Bauer disk diffusion method (4). Resistance breakpoints followed those outlined in the Clinical and Laboratory Standards Institute “Performance Standards for Antimicrobial Susceptibility Testing” (7). The bla-KPC gene associated with resistance to carbapenem antibiotics was screened by PCR following the protocol described by Hindiyeh et al. (23), as described in Table 2.

The multiple antibiotic resistance (MAR) index was calculated as the ratio of the number of antibiotics to which K. pneumoniae is resistant to the total number of antibiotics to which organism is was exposed (29). Index values were established as a risk assessment tool to determine whether bacterial isolates were from sources of high or low antibiotic use, whereby values greater than 0.2 indicate exposure to sources that have been highly exposed to antibiotics (10).

RESULTS

Positivity of K. pneumoniae in raw and RTE food. Overall, K. pneumoniae was detected in 21% (147 of 698) of raw and RTE food samples collected from across Singapore. A higher K. pneumoniae positivity rate was observed in raw food items (45%, 66 of 146) compared with RTE samples (15%, 81 of 552). K. pneumoniae was found in 58% (50 of 86) of raw vegetables (bean sprouts, spring onions, chilis, parsley, lettuce, cucumbers, carrots, and ginger) and 27% (16 of 60) of raw chicken and pork liver. Among RTE food samples, K. pneumoniae was detected in popiah or spring rolls (33%, 3 of 9), poultry dishes (27%, 14 of 52), mixed vegetable rice dishes (25%, 13 of 52), and porridge (25%, 3 of 12). Less than 20% K. pneumoniae positivity was observed for all other individual food types tested. The number of K. pneumoniae detected in specific food samples are shown in Table 1.

Virulence determinants detected among foodborne K. pneumoniae. Eight percent (11 of 147) of foodborne K. pneumoniae isolates were found to contain at least one (wcaG, K1, K2, or K54) of the eight virulence factors screened (wcaG and rmpA plasmid genes and K1, K2, K5, K20, K54, and K57 capsular types); the rmpA gene associated with liver abscess was not detected in K. pneumoniae isolates (0%, 0 of 147). Potentially virulent isolates were from both raw food (5 of 11; from chilis, lettuce, carrots, and pork liver) and RTE dishes (6 of 11; from a poultry dish, porridge, a noodle dish, a beverage, and a pork dish). Of the potentially virulent K. pneumoniae isolates, 3 of 11 had more than one virulence determinant: wcaG gene and the K1 capsular type were codetected in chilis and in lettuce, whereas wcaG and the K54 capsular...
type were codetected in a noodle dish. The \textit{wcaG} and \textit{K1}–positive \textit{K. pneumoniae} isolate was also MDR. The remaining potentially virulent \textit{K. pneumoniae} isolates conferred resistance to ampicillin (9 of 11) or no resistance to the antibiotics tested (1 of 11). Table 1 shows the virulence factors found among foodborne \textit{K. pneumoniae}.

**Antimicrobial resistance profiles of foodborne \textit{K. pneumoniae}**. Of the 97 foodborne \textit{K. pneumoniae} screened for antibiotic resistance, 10% (10 of 97) were MDR, i.e., resistant to at least three classes of antibiotics (reference 33; Table 1). Half (50%, 5 of 10) of these MDR \textit{K. pneumoniae} isolates were from raw chicken liver; the remaining MDR \textit{K. pneumoniae} isolates were from raw chilis (2 of 10), bean sprouts (1 of 10), pork liver (1 of 10), and a cooked poultry dish (1 of 10). In addition, 3 of 10 MDR \textit{K. pneumoniae} isolates had virulence determinants (Table 1). One MDR \textit{K. pneumoniae} isolate (from raw bean sprouts) was resistant to the third-generation cephalosporin (ceftiraxone).

The antibiotic resistance rates of \textit{K. pneumoniae} isolated from raw and RTE food are provided in Table 3. About 98% (95 of 97) of \textit{K. pneumoniae} isolates tested were resistant to ampicillin, and 14% (14 of 97) were resistant to tetracycline. Less than 10% resistance rates were observed for ciprofloxacin (8%, 8 of 97), chloramphenicol (7%, 7 of 97), sulfamethoxazole-trimethoprim (6%, 6 of 97), amoxicillin–clavulanic acid (5%, 5 of 97), nalidixic acid (2%, 2 of 97), amikacin (1%, 1 of 97), and ceftiraxone (1%, 1 of 97). No \textit{K. pneumoniae} isolates (0 of 97) showed resistance to gentamicin or norfloxacin.

Seventeen antimicrobial resistance phenotypes were observed among foodborne \textit{K. pneumoniae}. These patterns, along with corresponding MAR values that indicate association with sources heavily exposed to antibiotics, are summarized in Table 4. Most isolates (76%, 74 of 97) were resistant only to ampicillin, 11% (11 of 97) were resistant to antibiotics in two antibiotics classes, 10% (10 of 97) conferred resistance to three to six antibiotic classes (MDR), and 2% (2 of 97) were not resistant to the tested antibiotics. About 12% (12 of 97) of \textit{K. pneumoniae} isolates had a MAR index greater than 0.2.

**DISCUSSION**

This study reports that \textit{K. pneumoniae} from both raw and RTE food can be resistant to multiple antibiotics, can carry virulence genes, or is both antibiotic resistant and potentially virulent. It is worrying to find MDR and potentially virulent \textit{K. pneumoniae} in food that could be consumed raw (i.e., chilis). The observation of multidrug resistance among \textit{K. pneumoniae} from retail food types, and the presence of isolates associated with sources heavily exposed to antibiotics, is also concerning, because choices...
of antibiotic treatment may be challenging should these organisms cause infection. Our findings agree with reports of antibiotic-resistant \textit{K. pneumoniae} detected in various poultry and meat products (9, 21, 28, 38), indicating that \textit{K. pneumoniae} has the potential to become a reservoir of antibiotic genes in the food chain. Even though most (93\%, 136 of 147) isolated \textit{K. pneumoniae} did not have genetic virulence determinants, finding \textit{K. pneumoniae} isolates that carry antimicrobial resistance genes could potentially be an indirect public health hazard, regardless of their pathogenicity, because the available genetic pool of resistance is increased (8, 14). Retail meat has been cited to be a direct vehicle for the transmission of virulent, antibiotic-resistant \textit{K. pneumoniae} from food animals to humans (9), and in a study on emerging food safety issues related to meat, some outbreaks of increased severity were reported to be associated with etiological agents being antibiotic-resistant bacteria (13). MDR \textit{K. pneumoniae} isolates, and those heavily exposed to antibiotics, were from raw vegetables, as well as from chicken and pork liver. The MAR index observed in our study ranged between 0 and 0.5; 12 isolates had an index value greater than 0.2, suggesting that they had at some point come into contact with sources heavily contaminated with antibiotics.

Of the \textit{K. pneumoniae} isolated from food, about 8\% (11 of 147) harbored genetic factors associated with increased virulence. The \textit{rmpA} gene, a regulator of the mucoid phenotype that is closely correlated to community-acquired infection (50), was not detected. The \textit{wcaG} gene, associated with increased virulence by way of encoding sugar conversion to make \textit{K. pneumoniae} unrecognizable by macrophages triggering phagocytosis (2, 50), was observed in 6\% (9 of 147) of \textit{K. pneumoniae} isolates. The K54 capsular type reported to have extended spectrum \(\beta\)-lactamase activity and resistance to disinfectants (24) was also observed in a RTE food sample; this K54 capsular type was codetected with the \textit{wcaG} gene but was susceptible to all antibiotics except ampicillin. Capsular types (K1 and K2) that frequently coexist with aerobactin production (43, 50) and have been associated with nosocomial \textit{K. pneumoniae} liver abscess and bacteremia (17, 49, 42) were found on five occasions (3\%, 5 of 147).

Associations between liver abscess and gastrointestinal carriage of \textit{K. pneumoniae} have been reported in Asian countries, particularly in Taiwan (18, 30); similar associations were less common outside of Asia, and in the few accounts of \textit{K. pneumoniae} in the gut being linked to liver abscess, the cases were of Asian origin (30, 39). In a case series involving six Asian patients admitted to a U.S. hospital because of \textit{K. pneumoniae} liver abscesses, the gastrointestinal tract was suspected as the route of entry in one of the cases (34). In recent years, gastrointestinal carriage has also become recognized as a risk factor for \textit{K. pneumoniae} colonization in non-Asian cohorts. In Australia, for example, a study on likely \textit{K. pneumoniae} transmission events revealed that 48\% (13 of 27) of intensive care unit patients had gut colonization before infection (20).

In Taiwan and in Singapore, the characteristics of \textit{K. pneumoniae} liver abscesses are similar in genomic heterogeneity, serotype prevalence, and carriage of \textit{rmpA} (49). Various studies have suggested that geographic distribution patterns of virulent \textit{K. pneumoniae} subtypes may play a role in \textit{K. pneumoniae} liver abscesses, yet the sources and modes of \textit{K. pneumoniae} transmission remain unknown. Because \textit{K. pneumoniae} infections are preceded by colonization of the gastrointestinal tract and because microflora in food contributes to the gut microbiome, food could be speculated as a possible vehicle through which virulent \textit{K. pneumoniae} could enter the gastrointestinal tract. However, our data show that none of the \textit{K. pneumoniae} recovered from a range of food types carried the \textit{rmpA} gene, providing no evidence that the food samples were carriers of hypervirulent \textit{K. pneumoniae} strains linked to liver abscesses. In the absence of \textit{rmpA}, capsule synthesis is downregulated, and phagocytic resistance and the mucoid phenotype may be lost (49). Nevertheless, our detection of \textit{K. pneumoniae} strains belonging to the K1 capsular serotype, coexisting with the \textit{wcaG} gene, and in one instance, conferring multidrug resistance suggests that \textit{K. pneumoniae} may pose a public health risk and that its presence in food should not be underestimated. In addition, three \textit{K. pneumoniae} isolates from raw vegetables carried both \textit{wcaG} and the K2 capsular type and were resistant to multiple antibiotic classes (ampicillin-tetracycline-amikacin). Drug resistance is said to be associated with the spread of transmissible plasmids that also carry virulence genes, and the acquisition of resistant determinants can contribute to the persistence of virulent microorganisms (11).

For public health authorities, tackling the issue of antibiotic-resistant bacteria in the food supply chain requires concerted, interagency efforts. On an international
level, the WHO has outlined a global action plan to tackle antimicrobial resistance. Although it would be ideal to curb the spread of antibiotic resistance by a ban over antibiotic use in farming and food production, such extreme restrictions may be met with opposition. Increased cost of food production because of the reduction in total yield and growth size of animals, as well as the increased occurrence of animal diseases and foodborne infections (7, 12) when antibiotics are not used, is a concern. Thus, both administration and regulation of antibiotics need to be carried out with prudence. In Singapore, antibiotic use for food-rearing animals is strictly controlled. Antibiotics are allowed only for the treatment and prevention of diseases; antibiotic use for growth promotion is not allowed, and farmers importing to Singapore are required to comply with withdrawal periods specific to each antibiotic so that meat and meat products entering Singapore’s food supply contain only trace levels of or no antibiotics (1).

Being part of the normal animal and human flora, contamination of food with K. pneumoniae per se may not automatically constitute a public health risk. However, the presence of K. pneumoniae can be reflective of unhygienic food handling practices, undercooking, or postcooking contamination, especially when it is recovered from RTE cooked food. K. pneumoniae naturally occurs in soil and in farming; K. pneumoniae is of agricultural interest, because it has been demonstrated to increase crop yield in agricultural conditions (15). As such, its presence on raw vegetables is not surprising; the increasing use of organic fertilizers may also contribute to the presence of K. pneumoniae in food. Finding K. pneumoniae in raw food purchased at local markets and supermarkets indicates that additional food safety measures need to be practiced downstream, at retail and in the home. A study reported that lower K. pneumoniae prevalence rates on raw vegetables from wet markets, compared with those from hypermarkets, may have occurred because of quicker turnover of products (38), suggesting that prolonged storage of vegetables at humid conditions should also be avoided. Cooked RTE food, however, although safely cooked or packed, may be cross-contaminated with bacteria from raw products, meat juices, or other contaminated products or from food handlers with poor personal hygiene (46). The detection of K. pneumoniae in cooked RTE food may also result from its thermotolerance; although significant K. pneumoniae growth has been demonstrated at 60°C, complete inactivation of the bacteria may not always take place (37). At retail, K. pneumoniae may be spread through person-to-person (or person-to-item) contact during food preparation. Food handlers should thus be aware of the role they play in the spread (or control) of disease-causing bacteria. The study shows the importance of good hygiene and handling practices during food preparation to lower the risk of acquiring the potential pathogen.

Although lesser known to cause foodborne illness, the presence of MDR and potentially virulent (K1, K2, and K54 capsular serotypes and wcaG) K. pneumoniae in food suggests potential risk to public health, directly as a pathogen or indirectly as a vehicle of antibiotic genes that could be passed to other pathogens in the microflora of food and the human gut. The rmpA gene, known to increase the virulence of K1 K. pneumoniae and strongly associated with K. pneumoniae liver disease and community-acquired infection, was not found among isolates from food, providing no evidence to the link between food and hypervirulent K. pneumoniae associated with pyogenic liver abscesses.

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