Food Sources Harbor Antibiotic-Resistant Pathogens, Part 2

Antibiotic-resistant strains from food reservoirs at the local level can quickly disseminate, leading to worldwide public health risks

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Some foods serve as important reservoirs of antibiotic-resistant bacteria—from produce to seafood, livestock, and poultry, according to researchers who presented their findings during the 2015 ICAAC held in San Diego last September. These reservoirs expose food workers and consumers to pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum β-lactamase Escherichia coli (ESBL-E. coli), and carbapenem-resistant Enterobacteriaceae (CRE). All these pathogens may be carried asymptomatically or can cause difficult-to-treat infections, some confined to the gastrointestinal tract, while others cause more invasive disease. Due to blurring of boundaries between local and global markets, local food production practices can lead to regional and even worldwide public health risks when contaminated foods disperse resistant strains to distant locations, experts warn.

Furthermore, antibiotic-resistant strains may contaminate the hands of cooks or surfaces in kitchens and then transfer their resistance genes to other species of the human microbiota, generating resistance reservoirs within commensal flora of the gastrointestinal tract or on the skin and nasal mucosa. These reservoirs can foster dangerous ecologies within the gut, enabling commensal species to accumulate resistance genes and later pass them to transitory pathogens. Thus, enteric reservoirs can produce nosocomial pathogens with the potential to cause invasive infections in their immediate hosts or when passed to others in hospital or community settings. Researchers are investigating such reservoirs while developing strategies to reduce the threat that they pose to public health.

MRSA Strains in Livestock Reservoirs Can Be Transmitted to Humans

Farm animals are reservoirs for new strains of MRSA, according to a number of researchers who spoke during the ICAAC session “Animal-Human Transmission of Methicillin-Resistant Staphylococci.” Specifically, some dairy cows carry S. aureus isolates harboring the resistance gene mecC, according to Angela Kearns of Public Health England in London. “Animals can act as a reservoir and source for the emergence of novel methicillin-resistant S. aureus clones in human beings,” she says. “A bovine reservoir exists from which mecC MRSA is transmitted to people.”

According to her collaborators Robert Skov of the Statens Serum Institut in Copenhagen, Denmark, and Mark Holmes of the University of Cambridge, England, evidence from whole-genome sequencing (WGS) analysis substantiates that zoonotic link. “Phylogenetic analysis reveals two distinct farm-specific clusters comprising isolates from both the human case and their own livestock, with human and animal isolates from the same farm only differing by a small number of

SUMMARY

➤ Cows and pigs are major reservoirs for novel methicillin-resistant Staphylococcus aureus, which are promiscuous colonizers, adept at moving among diverse host species.
➤ Carbapenem-resistant Enterobacteriaceae (CREs) are becoming a major public health concern, having recently infiltrated wild-caught and farm-raised food sources.
➤ Routine commercial activities within the global economy can help to disseminate antibiotic resistant clones and genes far and wide.
➤ Foods carrying drug-resistant bacteria can pass resistance genes to commensal bacteria in the gut, converting an individual’s microbiota into a resistance reservoir.
single-nucleotide polymorphisms (SNPs), which supports zoonotic transmission,” they note. Their study exemplifies WGS as being a valuable tool in clinical epidemiology and source tracking.

These livestock-associated MRSA strains are promiscuous colonizers, adept at moving among diverse host species. One mecC MRSA clone, ST130, is now dispersed among a wide range of domestic and wild animals, including birds, seals, otters, hedgehogs, ungulates, rodents, carnivores, and other, more-exotic species in the Copenhagen zoo. “The potential for zoonotic transmission of S. aureus between livestock, companion animals, and humans has been exemplified by the emergence of these strains,” says Skov, who points to its clonal spread in Denmark. Moreover, Holmes adds, its establishment within these diverse animal reservoirs suggests a potential for further geographic expansion. While human clinical cases of ST130 MRSA are limited primarily to rural areas in the UK and Denmark so far, human and animal carriers are beginning to deliver this same resistant clone to other, more-urban settings, they note.

The mecC resistance gene may also be transmitted among other staphylococcal species that are native to livestock or wild animals, according to Holmes. “MecC has also been detected in other species of staphylococci, specifically Staphylococcus xylosus from bovine mastitis and Staphylococcus stepanovicii from a wild European lynx,” he says. The former may contaminate milk collected from dairy cows, potentially exposing humans if the milk products they consume are not pasteurized.

Pig farms are also hotspots for MRSA strains, says Kearns. “A survey of slaughter pigs in the Netherlands showed that 39% harbor MRSA ST398, and another survey showed that 27% of people working at, or living on, a livestock farm in the Netherlands carry that strain,” she says. Meanwhile, data collected in Denmark suggest that these rates can go much higher, with as many as 88% of pigs testing positive at slaughter, according to Skov. Farm workers carrying these strains may then introduce them into the general population or, alternatively, butchered animals may yield food products that carry and transmit those strains. In 2015, some UK pork products were found to be contaminated with MRSA strain CC398, exposing consumers and food workers to this pathogen.

Foods Carrying Carbapenem-Resistant Enterobacteriaceae Put Consumers at Risk

CREs are becoming a major public health concern, having recently infiltrated wild-caught and farm-raised food sources. In particular, ESBL and carbapenemase-producing Escherichia coli, Salmonella, Acinetobacter, and Campylobacter species are being found in livestock and poultry species, including cattle, pigs, sheep, and chicken, as well as in eggs, dairy products, and produce, according to Edward Topp of Agriculture and Agri-Food Canada in London, Ontario, who spoke during the ICAAC session “Surrounded by the Enemy? The Environment and Foodstuffs as Sources or Reservoirs of Antimicrobial Resistance Threats.”

Agriculture sector-based surveillance of CREs is spotty, with most reports originating from European countries. However, limited data from Australia, Japan, the United States, and Europe point to similar trends, Topp says—citing, for example, Beatriz Guerra of the Federal Institute for Risk Assessment in Berlin, Germany. “There is a need for intensified surveillance on the occurrence of carbapenemase-producing bacteria in the food chain and other animal sources in order to assist in the formulation of measures to prevent their potential spread,” she notes.

Other investigators echo Guerra. “No surveillance system incorporates selective media monitoring for CREs routinely,” says David Boyd of the Public Health Agency of Canada in Ottawa, who spoke in the ICAAC session “Emergence of Resistance: Environmental and Food Chain Threats.” He and his colleagues, who seek to develop an international standard for detecting CREs in the food chain, are monitoring seafood that is imported into Canadian markets. Clams and shrimp from Vietnam harbor Enterobacteriaceae species with clinically concerning β-lactamases and carbapenemases, they report. Similarly, wild-caught seafood from China and Korea carry bacteria harboring the carbapenem resistance gene blaOXA-48, Topp adds (Fig. 1).

Various kinds of seafood can be sources of novel resistance genes, according to Boyd, who identified a novel carbapenemase within Vibrio cholerae species in frozen shrimp from India. The gene, designated blaVCC-1, is 59% identical to class A carbapenemases, defined by their serine-based hydrolytic mechanisms. It confers a resistance profile similar to other well-known and
clinically threatening members of the imipenemase/non-metallocarbapenemase group, such as KPC (*Klebsiella pneumoniae* carbapenemase) and IMI (imipenem-hydrolyzing β-lactamase).

**Food Commerce Disseminates Antibiotic Resistance Factors**

These reports highlight how routine activities within the global economy can help to disseminate antibiotic-resistant clones and genes far and wide. Foods that are cultivated or caught in remote locations are transported to urban centers throughout the world, exposing vast numbers of people to whatever antibiotic-resistance factors they might contain. Meanwhile, food production practices in one country or region can influence public health risks locally as well as globally, says Topp.

For example, antibiotic use in livestock and poultry production correlates with meat products from those herds carrying resistant bacterial strains, according to Topp from Canada. Specifically, ceftiofur use in agriculture during the last decade parallels levels of cephalosporin-resistant *Salmonella* found in chicken meat sold at retail levels, he says. When such drugs were withdrawn from agricultural use in 2005, the levels of cephalosporin-resistant strains in such foods declined substantially, followed by a subsequent increase when the drugs were reintroduced a few years later.

Similarly, when enrofloxacin was licensed for animal use in 1993, resistant isolates in livestock steadily increased, Topp continues. In both cases, fluctuations in resistance in foodborne *Salmonella* paralleled those found in human clinical isolates, suggesting that increased exposure of consumers and food handlers to these resistant strains is associated with their appearance in cases of human disease.

Paula Di Carlo of the University of Palermo, Italy, who spoke in the same session as Boyd, also reports a link between resistant strains found in food supplies and human clinical cases. For instance, ESBL-*E. coli* strains isolated from patients with bloodstream infections were of the same clonal lineage as those found in broiler chickens.
within the same region of Italy, she found. “Detecting this subclone in chicken meat samples can support the hypothesis of its role as a contributor to the wide prevalence of fluoroquinolone-resistant (FQ-R) and ESBL-producing E. coli as etiological agents of human infections in our geographic area.”

Specifically, the ST131 H30 subclone is an invasive, multidrug-resistant strain that emerged recently as a global threat to public health. Its clonal expansion and dissemination may in part be due to food production and distribution, according to Di Carlo. “This study provides support for the hypothesis that food products of animal origins, especially broilers, may be a reservoir for E. coli ST131,” she says.

Antibiotic-resistant bacterial strains can also be transmitted via produce, particularly when animal manure is used to fertilize crops, according to Topp. “Fecal material is enriched for antibiotic-resistant bacteria and soil fertilized with these materials becomes enriched with these genes,” he says. “This represents a large reservoir for crop exposure that must be managed.” Indeed, ESBL bacteria were detected on diverse vegetables and fruits, including carrots, celery, spinach, pears, and peaches. Able to survive for prolonged periods on and within food products, these resistant strains persist from harvest to market, having been documented, for instance, in bagged, ready-to-eat spinach.

While some consumers prefer organic produce, such production methods offer no respite from antibiotic-resistant bacterial strains, Topp says, referring to work by Raymond Ruimy of Nice Academic Hospital, France. “[A] large, year-long constructed sample set containing 399 products showed that, irrespective of their mode of production (organic vs. conventional), raw fruits and vegetables are heavily contaminated by gram-negative bacteria resistant to multiple antibiotics,” Ruimy reports. “Of the products tested, 13% carried bacteria producing ESBLs.” Further, produce grown in or on the ground, such as radishes or strawberries, is more than twice as likely to harbor resistant strains as those grown above it, due to increased contact with soil, he reports.

Irrigating crops with water carrying multidrug-resistant (MDR) pathogens can also give rise to public health challenges, according to Amy Sapkota of the University of Maryland. For example, a 2005 outbreak of antibiotic-resistant Salmonella was traced to contaminated irrigation water used on tomato crops, she says. Similarly, contaminated irrigation waters can introduce MDR enterococci onto tomato fruits and leaves, she says. “E. faecalis disperse efficiently within a farm setting, transferring from one habitat to another such as from water and soil to tomato leaves and fruit.” Prolonged droughts may elevate such risks, when reclaimed water resources, which could carry MDR clinical strains, are used to irrigate crops (Fig. 2).

Resistant strains deposited on produce may transfer resistance genes to nearby microbes living on vegetation, seeding additional resistance reservoirs, says Topp, citing research by Eva Raphael of the University of California, Berkeley. “Saprophytes in common fresh produce can harbor drug resistance genes that are also found in internationally circulating strains of gram-negative bacterial pathogens; such a source may thus serve as a reservoir for drug resistance genes that ultimately enter pathogens to affect human health,” she says. Thus, food may directly expose consumers to antibiotic-resistant pathogens or may face them with innocuous bacterial species carrying resistance genes that then can be transmitted to more virulent microbial species and strains. “Food is enriched for non-pathogens that carry antibiotic-resistance genes, which represents a potentiated reservoir for resistance gene recruitment into pathogens,” says Topp.

Drug-Resistance Reservoirs within Our Gut Microbiota

After an individual consumes foods carrying drug-resistant bacteria, ingested microbes that survive the rigors of the upper gastrointestinal tract can pass mobile resistance genes to commensal bacteria in the gut, converting the microbiota into a resistance reservoir. Alternatively, when individual patients are treated with antibiotics, resistance reservoirs may also arise within the microbiota. In either case, the reservoir can persist, lying dormant until an opportunistic pathogen appears, at which point resistance factors can move from an innocuous commensal species into the newly arriving pathogen.

The commensal species, Enterobacter cloacae, can serve as a reservoir for carbapenem resistance genes, according to Timothy Johnson of the University of Minnesota, who spoke in the session “Resistance Rush-Hour Traffic: How
Do Resistance Genes Travel? “E. cloacae appears to be emerging in the upper Midwestern United States as a prominent carrier of KPC,” he says.

Examining a recent outbreak in Minnesota and North Dakota, Johnson finds that the KPC resistance gene is easily transferred among E. cloacae strains within the human gut, which transmit between patients, resulting in widespread dissemination across hospitals. Even more alarming, the carbapenem resistance plasmid can be transferred to other bacterial species, yielding extremely dangerous MDR pathogens in some cases. In one patient, the plasmid was transferred from E. cloacae to E. coli ST131, a pathogen of global importance. “This specific example highlights a dangerous scenario in which a circulating, MDR clone (E. cloacae) has transferred its plasmid to a globally emergent extraintestinal pathogen (E. coli ST131), rendering it nearly pan-resistant to medically relevant antibiotics,” he says.

Johnson warns that CP-E. cloacae are likely circulating among asymptomatic carriers in the population, serving as a reservoir for resistance that may be transferred to other fecal flora or transient pathogens at any time. E. coli ST131 may be a particularly salient threat, as it is a strong enteric colonizer that can also pass asymptptomatically through the population and therefore has a high likelihood of coming into contact with CP-E. cloacae. “Extensively drug-resistant clones are emerging in response to selective pressures, and many of these types of clones currently may be circulating worldwide,” he says. “Increased surveillance for such clones is an essential step toward understanding mechanisms of dissemination and informing appropriate infection prevention and control measures.” The Centers for Disease Control and Prevention considers CP-CRE as an urgent threat, and such strains have been detected in the US, Greece, Spain, and Brazil.

Colonizing enterococci also provide a melting pot for resistance and virulence genes that later can transfer to neighboring bacteria, according to Guido Werner of the Robert Koch Institute in Berlin, Germany. Enterococci can harbor multi-
ple resistance plasmids simultaneously, which can then consolidate, inserting resistance genes from different plasmids onto a single plasmid backbone. The resulting multifunctional mega-plasmids can confer full arsenals of resistance and virulence genes. For example, the 28-kb pLG1 plasmid in vancomycin-resistant enterococcal strains also carries resistance to erythromycin, bleomycin, and heavy metals, as well as many virulence genes.

Multifunctional megaplasmids generated in enterococci, or pathogenicity islands encoded within them, can be transferred horizontally within the enterococcal population and to other bacterial species within the gut, Werner says. Vancomycin-resistance clusters of enterococci may originate from commensal microflora, including strains of clostridium and ruminococcus species, and then can transfer MDR megaplasmids to opportunistic pathogens such as *S. aureus* or to other enterococci species, increasing the pathogenic potential of facultative bacteria, making these relatively harmless bacteria far more dangerous strains.

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