Research Note

Antibiotic Resistance and Growth of the Emergent Pathogen *Escherichia albertii* on Raw Ground Beef Stored under Refrigeration, Abuse, and Physiological Temperature

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**ABSTRACT**

*Escherichia albertii* is an emerging gram-negative facultative rod that has been implicated in multiple cases of human diarrheal disease, particularly in young children. When biochemical and other typing methods have been used, this organism has often been misidentified due to similarities with other members of the family *Enterobacteriaceae*. Isolates have been reported to be capable of producing attachment and effacement lesions via the synthesis of intimin, cytolethal distending toxin, and a variant form of Shiga toxin. The purposes of this study were to characterize the antibiotic resistance characteristics and the growth of individual strains of *E. albertii* on raw ground beef at different storage temperatures. Nalidixic acid–resistant strains of *E. albertii* were inoculated onto raw ground beef to a target of 4.0 log CFU/g, and samples were then aerobically incubated at 5, 22, or 35°C for various time periods prior to microbiological enumeration of the pathogen on lactose-free MacConkey agar containing 50 mg of nalidixic acid per liter and 0.5% l-rhamnose. Antibiotic resistance was determined using a broth microdilution assay. *E. albertii* did not grow at 5°C, with populations declining slowly over 14 days of refrigerated storage. Strains of the organism grew well under abusive storage, increasing by 2.5 to 3.1 log CFU/g and 4.1 to 4.3 log CFU/g after 24 h at 22 and 35°C, respectively. All strains were resistant to tetracycline but were sensitive to tested cephalosporins and chloramphenicol. Resistance to penicillin was observed, but susceptibility to other members of the β-lactam group, including ampicillin, amoxicillin, and clavulanic acid, was recorded. *E. albertii* represents an emerging pathogen with a probable foodborne transmission route. Future research should focus on verifying food process measures able to inactivate the pathogen.

*Escherichia albertii* is a gram-negative rod that may represent an emergent foodborne enteric pathogen based on recognition of its ability to form attachment and effacement lesions via the synthesis of intimin (10), cytolethal distending toxin (13), the recent isolation of strains carrying genes encoding a variant of Stx2 (14), and its previous recovery from stool samples from Bangladeshi children exhibiting diarrheal disease (2, 3). Oaks et al. (13) isolated the organism from migratory fowl and poultry, while Lindsey et al. (11) reported recovery of 62 putative positive isolates from rinsates obtained from 1,644 poultry carcasses. Multiple strains of the microbe have been identified and characterized by various means including both genetic and biochemical marker assays, revealing similarities of *E. albertii* to *Escherichia coli* and *Shigella flexneri* (1, 6, 7). Use of biochemical profiling systems has resulted in the misidentification of *E. albertii* as *Hafnia alvei*, *Yersinia ruckeri*, and *E. coli* (1). Previous studies have reported that isolates are nonmotile at 35°C, oxidase negative, and negative for citrate, indole, and acetoin from fermented weak acid (e.g., lactic acid) (1, 10, 17). The microbe ferments acid and gas from d-glucose and acid from d-fructose, d-galactose, and d-mannitol (1). Stock et al. (17), however, reported that isolates were unable to ferment l-rhamnose and d-xylose. Isolates have been reported to not ferment lactose and are not known to synthesize β-d-glucuronidase (14).

Huys et al. (7) reported that strains of *E. albertii* were resistant to penicillin and tetracycline but susceptible to chloramphenicol. Sensitivity of isolates to aminoglycosides, various cephalosporins, and quinolones has also been reported (17). With respect to resistance to food processing measures, the organism has been reported to be significantly more sensitive to low pH environments than wild-type *E. coli* O157:H7 as determined by plating survivors following incubation in acidified medium (15). The organism demonstrates decreased resistance to high hydrostatic pressure treatment and *D* ~ ~ values that range from ~2 to 6 min less than those for *E. coli* O157:H7, *S. flexneri*, or *Shigella boydii* (15).

To date there have been only a very small number of studies detailing the ability of *E. albertii* to survive and grow on foodstuffs under various conditions, despite its previous recovery from poultry carcasses (11). Sharma et al.
demonstrated the ability of individual strains to thrive on fresh-cut lettuce at 30°C, their population increasing 3 to 4 log following 48 h of aerobic incubation. Incubation at 5°C, however, inhibited growth, with populations of the pathogen remaining unchanged over 8 days of incubation (16). Rapid growth was observed on ground turkey inoculated with 4.0 log CFU/g of three strains of E. albertii; a 4.0-log cycle increase in numbers of each strain of the pathogen was observed following 48 h of aerobic incubation at 30°C (20). Populations of E. albertii were, however, unchanged after 14 days at 5°C. Therefore, the objectives of the study reported here were to determine the ability of individual strains of E. albertii to resist antimicrobials in vitro and to survive or grow on raw ground beef under conditions of refrigeration and moderate and extreme temperature abuse.

**MATERIALS AND METHODS**

**Bacterial strains and recovery medium.** Nalidixic acid-resistant (Nal') spontaneous mutants of E. albertii ATCC 10457, 19982, and 9194 (referred to as E. albertii 457, 982, and 194, respectively) were generously donated by Dr. Manan Sharma, U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS), Beltsville, MD. Immediately upon receipt of strains, isolates were resuscitated in tryptic soy broth (TSB; BD, Franklin Lakes, NJ) via two successive transfers followed by aerobic static incubation at 35°C. Strain purity, antibiotic resistance, and inability to ferment D-xylose were confirmed by streaking a loopful of overnight culture on MacConkey agar base (BD) containing 10 g of D-xylose per liter (Alfa Aesar, Ward Hill, MA) and 50 mg of Nal per liter (Sigma-Aldrich, St. Louis, MO) (R-MAC-N). E. albertii survivors were enumerated throughout all experiments on R-MAC-N and were identified as colonies displaying hydrolysis of bile salts and no fermentation of D-xylose following 24 h of aerobic incubation at 35°C.

**Antibiotic resistance determination.** Isolates of E. albertii were prepared and submitted to the Texas Veterinary Medical Diagnostic Laboratory (College Station) for determination of antibiotic resistance in addition to Nal. Antibiotic resistance was determined using a 96-well assay platform with the Vizion Sensititre System (Trek Diagnostic Systems, Thermo-Fisher Scientific, Inc.). Antibiotic resistance susceptibility or resistance status was determined using Clinical and Laboratory Standards Institute (CLSI) veterinary-specific interpretive criteria or, when these were not available, human interpretative criteria. These studies were completed in order to aid further studies determining optimal antimicrobials for incorporation into selective enrichment and/or selective or differential enumeration media.

**Preliminary trials.** Prior to the initiation of growth experiments, raw ground beef (85% lean) was purchased from a local retail grocery and immediately returned to the Food Microbiology Laboratory, Department of Animal Science, Texas A&M University, College Station. Two 11-g samples were aseptically diluted in 99 ml of 0.1% peptone diluent (PW; Thermo-Fisher Scientific, Waltham, MA) and stomached for 1 min at 230 rpm, and survivors were enumerated via spread plating of sterile petri dishes containing tryptic soy agar (TSA; BD), followed by aerobic incubation of plates at either 35°C for 24 h or 5°C for 7 days for the estimation of aerobic mesophiles and psychrotrophs, respectively. Results were utilized in the assessment of microbiological quality of the foodstuff and the determination of experimental serial dilution schemes. Homogenate of beef and PW were also streaked onto surfaces of R-MAC-N–containing petri dishes in order to verify that no background microbiota organisms resistant to Nal and unable to utilize D-xylose were detected.

**Beef inoculation and incubation.** Following the completion of preliminary studies to estimate beef microbiological quality, freshly ground beef (85% lean) was purchased from the same retail market used for preliminary studies and prepared for inoculation and incubation. On the day of beef purchase, aliquots of beef were inoculated with 6.0 log CFU of E. albertii 457, 194, or 982 per ml, separately. Following plating on day zero, samples stored at 5°C were pulled for enumeration on days 3, 7, and 14, whereas those stored at 22°C were enumerated after 1, 3, and 5 days of storage. Finally, samples stored at 35°C were removed from incubation and processed for E. albertii enumeration on days 1, 2, and 3 postincubation.

**Microbiological assays.** After the intervals given above, 11-g beef samples were removed, mixed with 99 ml of 0.1% PW, and homogenized via hand massaging for 1 min prior to preparation of serial dilutions and plating of survivors. All dilutions were prepared in 0.1% PW. E. albertii was then spread plated on R-MAC-N, while aerobic mesophiles and psychrotrophs were plated onto plate count agar (BD). Surviving E. albertii and mesophilic aerobes were enumerated following 24 h of aerobic incubation at 35°C, whereas psychrotrophic aerobes were enumerated following 7 days of aerobic incubation at 5°C (12, 21). E. albertii organisms were identified as colonies displaying phenotypes of Nal', precipitation of bile, and nonutilization of D-xylose.

**Statistical analysis.** All growth experiments were completed in triplicate, and means were produced from like samples. Sample means were tested for significant differences as a function of incubation temperature, E. albertii strain, and days of incubation using a one-way analysis of variance (ANOVA) with means separated completed with Fisher’s least significant differences procedure (P < 0.05) using SPSS v. 16.0 (SPSS, Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

Antibiotic resistance profiles of E. albertii 982, 194, and 457 are presented in Table 1. Similar to results reported by Stock et al. (17), E. albertii isolates demonstrated resistance to penicillin but were sensitive to other members of the penicillins, including ampicillin, amoxicillin, and clavulanic acid. Isolates demonstrated susceptibility to the cephalosporins, although E. albertii 457 showed intermediate sensitivity to cefoxitin. Isolates were sensitive to chloramphenicol and clindamycin, again similar to findings previously reported (17). Whereas isolates were susceptible to trimethoprim-sulfamethoxazole, tetracycline resistance was observed for all strains tested, in like fashion to findings reported by others (7, 9) (Table 1). Isolates in the present study demonstrated resistance to three of eight tested antimicrobials identified by the World Health Organization (WHO) as being of critical importance in the treatment of human diseases by bacterial pathogens, those being erythromycin, the β-lactam penicillin, and tetracycline (22). Additionally, E. albertii 457 demonstrated resistance to cephalothin and oxacillin, identified by the WHO as
Table 1: Resistance and susceptibility of E. albertii isolates to antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)</th>
<th>982</th>
<th>194</th>
<th>457</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≤4.00</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin–clavulanic acid</td>
<td>≤4.00</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8.00</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>≤8.00</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>16.00</td>
<td>S</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>≤2.00</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>0.50</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8.00</td>
<td>I</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&gt;1.00</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>≤0.50</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&gt;4.00</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤1.00</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>0.50</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Orfloxacin</td>
<td>2.00</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Oxacillin + 2.0% NaCl</td>
<td>&gt;4.00</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&gt;8.00</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;8.00</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≤0.50</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*Antibiotic resistance was determined using Sensititre Vizion broth dilution assay (Trek Systems, Thermo-Fisher Scientific, Inc.), with breakpoint interpretations made using veterinary-specific criteria or, when required, human-specific criteria. S, susceptible; R, resistant; I, intermediate.

aLowest concentration relative to interpretative breakpoints whereby all E. albertii strains were identified as being sensitive, intermediately resistant, or resistant to antibiotic.

Highly important in human disease treatment (22). The resistance and susceptibility patterns of E. albertii for the WHO’s list of critically important antibiotics were similar to those reported by the Centers for Disease Control and Prevention (CDC) for E. coli O157:H7 human isolates, with notable exceptions. Whereas >90% of CDC-tested E. coli O157:H7 isolates were resistant to 4.0 µg of amoxicillin–clavulanic acid per ml, E. albertii isolates were consistently sensitive at the same concentration (5). However, E. albertii isolates demonstrated resistance to tetracycline at concentrations similar to those at which E. coli O157:H7 isolates were also resistant (5). The antibiotic resistance of E. albertii was similar to that of Shigella as observed by the CDC, with the exception that E. albertii was sensitive to 4.0 µg of amikacin per ml, while most isolates (72.5%) of Shigella exhibited resistance at this concentration (5). Thus, E. albertii antimicrobial resistance profiles may differ from those of other species within the genus Escherichia and the family Enterobacteriaceae, despite having some similarities in fermentative pathways that may lead to misidentification by biochemical testing methods.

Prior to inoculation, numbers of aerobic and facultatively anaerobic psychrotrophs naturally present on beef were consistently below the limit of detection (~1.0 log CFU/g) across replications (data not shown). Preliminary completion of aerobic plate counts indicated that mesophilic aerobes were present on beef at an average of 3.3 ± 0.5 log CFU/g. No naturally occurring NaCl non–l-rhamnose-fermenting colonies were detected following streaking of beef and PW homogenate on surfaces of R-MAC-N medium. Populations of E. albertii strains did not increase over 14 days of storage at 5°C, declining by 1.7 to 2.3 log CFU/g over this storage time (Fig. 1A). The lack of growth, coupled with the slow decline in populations during storage at 5°C, is not unexpected, given similar findings reported previously that indicated that Escherichia spp. do not grow at this temperature (9, 19). Tamplin (18) reported that E. coli O157:H7 added approximately 2.5 to 3.0 log CFU/g to its numbers over 150 h at 10°C in nonsterile ground beef. At 22°C, a 3.7- to 4.0-log increase was observed over 3 days for E. albertii isolates in this study. This also is not surprising given reports by Ingle et al. (9) that E. albertii does not differ from other species of Escherichia with respect to rate of growth and optimal growth temperature range and reports by others that E. coli exhibits significant multi-log-cycle growth with potentially rapid (~2 h) onset of growth at 20 to 23°C (4, 8). Indeed, though statistical differences were observed in populations of E. albertii strains after 24 h at 22°C (P < 0.05), there was no significant difference (P ≥ 0.05) in their ability to add numbers over the overall 5 days of incubation at 22°C (Fig. 1B). Likewise, the significant (P < 0.05) addition of E. albertii to its numbers at 35°C (4.0 to 4.3 log CFU/g after 24 h) compared with 5 and 22°C confirms previous reports of the organism’s ability to replicate efficiently on meat surfaces at temperatures of ≥30°C (Fig. 1C) (20). Statistical analysis of E. albertii populations indicated that strains of E. albertii did not significantly differ with respect to their ability or inability to add to their numbers on ground beef as a function of incubation temperature (P ≥ 0.05).

In conclusion, E. albertii represents a likely challenge to the safety of foods for human consumption, especially undercooked meat and poultry products, or potentially fresh produce items that become cross-contaminated via irrigation waters that are contaminated with faecal matter from wild fowl. Isolates of the organism continue to be misidentified by standard typing assays, and some have reported isolates capable of synthesizing Shiga toxin and cytolethal distending toxin, as well as bearing multiple subtypes of intimin, essential to the formation of attachment and effacement lesions in the human gut (14). Isolates tested in this study were not screened for genes encoding Shiga toxin but were described as possessing eaeA genetic elements. A system of testing that combines the use of multiple screening and characterization technologies, including biochemical and/or molecular assays, would likely be more effective for consistent differentiation of the pathogen from other organisms in food or clinical testing, thus leading to more accurate quantification of disease burden borne by E. albertii. Nevertheless, the organism’s inability to grow under refrigeration (5°C)
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