Characteristics of Strains of *Escherichia coli* Isolated from Locally-Fermented Milk ("Nono") in Zaria, Nigeria

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**ABSTRACT**

Of a total of 200 fermented milk ("Nono") samples collected, 79 (40%) were positive for *Escherichia coli*, all isolates being of fecal origin. The mean pH was 4.41 and the values ranged from 4.03 to 4.81. Thirty-three (42%) isolates produced alpha hemolysin while two (3%) had beta hemolytic pattern. Eight (26%) of 31 randomly selected isolates produced heat-labile (LT) enterotoxin as detected by the rabbit ileal loop test. On testing the susceptibility of 50 isolates to antimicrobial agents, resistance was high to nitrofurantoin (100%), furadantoin (98%), Co-trimoxazole (98%), tetracycline (90%), and sulphafurazole (98%). Only 17 (34%) and 16 (32%) isolates were resistant to gentamycin and colistin sulfate, respectively. It was concluded that *E. coli* of fecal origin in "nono" could be a health risk as gastro-enteritis may result. Furthermore, the presence of other enteric pathogens cannot be ignored.

Although *E. coli* has long been recognized as a normal inhabitant of the gastro-intestinal tract of man and animals, on several occasions it has been responsible for acute gastro-enteritis, diarrhea, and extra-intestinal infections (30). Enterotoxigenic strains of *E. coli* (ETEC) have been associated with epidemic and sporadic outbreaks of infantile diarrhea in humans and some diarrheal diseases in domestic animals (3,26). Although some reports exist on the enterotoxigenicity of *E. coli* strains isolated from animals (5) in Nigeria, information is not available on food isolates.

*E. coli* has been isolated from water as a fecal contaminant and from various foods and food products of animal origin including market raw milk (2,6,8,25). The organism has also been implicated in a number of foodborne and waterborne outbreaks worldwide (24,26). Of the various locally-produced food products in Nigeria, only a report exists on a study of *E. coli* contaminants on 'Suya', a roasted beef product (14).

The antibiograms of isolates of *E. coli* from various sources to antimicrobial agents have been reported (15,22). Also, infective drug resistance in both human and animal strains is now known to be widely distributed and transferrable from pathogenic to non-pathogenic *E. coli* and other enteric organisms (20). Some studies have been reported on antibiograms of *E. coli* strains from animals in Nigeria (12,21) but little is known about those isolates from locally-prepared foods.

Therefore, this study was designed to determine the prevalence of *E. coli* in a popular locally-fermented milk product ("nono"), and to determine some of the characteristics of the isolates.

**MATERIALS AND METHODS**

**Product: 'Nono'**

"Nono" is a local uncontrolled fermentation product of cow's milk which forms a major part of the staple food of a high proportion of the populace in northern Nigeria. It is produced mainly by the nomadic Fulanis.

**Preparation of 'Nono'**

First, fresh milk is directly obtained from a cow into a properly washed and sun-dried calabash. The milk is then kept wide open in the sun for approximately 2 h to facilitate separation of the fat layer. Thereafter, some amount of the overnight fermented milk is added to serve as starter culture. A large volume of water is added before the inoculated fresh milk is left overnight at room temperature for fermentation.

A variation in the method practiced by the settled fulanis is the heating of the milk for 20-30 min, followed by a cooling period of 2-3 h to replace the habit of simply keeping milk for 2 h under the sun.

**Sale of 'Nono'**

Regardless of the method of preparation, prior to sale, a common practice among the female maiden hawkers is the fraudulent addition of stream water and other miscellaneous products such as the milky-white supernatant of water-soaked baobab tree seeds, to the fermented milk. All these are done to increase the volume of the product and an attempt to improve the taste and color.

**Source of samples**

A total of 200 fermented milk products were bought at seven markets in Zaria and environs (Table 1). From each 'nono'
**TABLE 1. Prevalence of fecal E. coli in ‘nono’ and pH values.**

<table>
<thead>
<tr>
<th>Market</th>
<th>Number of samples collected</th>
<th>Number (%) positive for E. coli</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kongo</td>
<td>12</td>
<td>0 (0.0)</td>
<td>4.48</td>
<td>4.37-4.60</td>
</tr>
<tr>
<td>Panladan</td>
<td>10</td>
<td>3 (30.0)</td>
<td>4.53</td>
<td>4.31-4.81</td>
</tr>
<tr>
<td>Sabon Gari</td>
<td>25</td>
<td>12 (48.0)</td>
<td>4.38</td>
<td>4.21-4.64</td>
</tr>
<tr>
<td>Samaru</td>
<td>67</td>
<td>30 (44.8)</td>
<td>4.37</td>
<td>4.03-4.52</td>
</tr>
<tr>
<td>Shika</td>
<td>38</td>
<td>9 (23.7)</td>
<td>4.40</td>
<td>4.11-4.62</td>
</tr>
<tr>
<td>Tudun-Wada</td>
<td>36</td>
<td>18 (50.0)</td>
<td>4.55</td>
<td>4.25-4.71</td>
</tr>
<tr>
<td>Zaria City</td>
<td>12</td>
<td>7 (58.3)</td>
<td>4.40</td>
<td>4.21-4.51</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>79 (39.5)</td>
<td>4.41</td>
<td>4.03-4.81</td>
</tr>
</tbody>
</table>

*All 79 isolates of E. coli were of fecal origin.*

**pH determination**

The pH of every ‘nono’ sample was determined using a digital pH meter (Kent Digital pH meter, U.K.).

**Isolation of E. coli**

Approximately 15 ml of each sample of ‘nono’ were centrifuged in sterile screw cap tubes at 2,500 to 2,500 rpm for 15 min. The supernatant was decanted and the precipitate was streaked for isolation on eosin methylene blue (EMB) plates. Incubated colonies that appeared blue-black with transmitted light and with greenish metallic sheen by reflected light were picked, streaked on nutrient agar (NA) slants, and incubated overnight at 37°C. Cultures on NA slants were stored at 4°C till needed.

**Identification of E. coli**

Isolates of E. coli were identified using standard techniques (9).

**Detection of fecal E. coli**

The membrane filter technique described by Lacheballier et al. (19) was used with a slight modification. Isolates were streaked directly onto E.-agar (Difco, Detroit, MI, U.S.A.) in 47 mm plastic petri dishes. The plates were then incubated upright in a water-bath at an elevated temperature of 44.5°C for 24 h. Light-blue to dark-green colonies on E.-agar medium were regarded as fecal coliforms as previously suggested (31).

**Detection of hemolysin**

Five-percent sheep blood agar plates were streaked with isolates and incubated at 37°C for 24 h. Patterns of hemolysis around isolated colonies were observed and recorded as alpha for incomplete clearing, beta for complete clearing, and gamma for no evidence of hemolysis.

**Susceptibility of isolates to antimicrobial agents**

**Antimicrobial agents.** Antibiotic discs (Oxoid multidiscs) and concentrations used were: tetracycline (50 μg), gentamycin (10 μg), sulphafurazole (500 μg), co-trimoxazole (25 μg), furadantoin (25 μg), ampicillin (25 μg), kanamycin (30 μg), nitrofurantoin (200 μg), and colistin sulphate (10 μg).

**Susceptibility testing.** The tests were conducted by the disc diffusion method of Bauer et al. (7). The antimicrobial agent discs were placed on inoculated Mueller-Hinton agar plates. The inverted plates were incubated aerobically at 37°C for 16 to 18 h. Zones of inhibition, to the nearest millimeter, were interpreted as susceptible, intermediate, and resistant based on the interpretative table recommended by the disc manufacturer.

**Growth of isolates for enterotoxin production**

Thirty-one randomly selected isolates of E. coli were inoculated into 20 ml of brain heart infusion (BHI) broth in conical flasks, which were subsequently incubated for 18 h on a gyratory shaker with 20 rotations per minute at room temperature. The growth resulting was spun down in a refrigerated centrifuge at 14,000 rpm for 20 min and the supernatant collected.

**Detection of heat labile (LT) toxin**

To detect heat labile enterotoxin, the procedure described by Adetosoye (3) using the rabbit ileal loop test was employed.

**RESULTS**

The frequency of isolation of E. coli from ‘nono’ bought from seven different markets is shown (Table 1). The pH values of these are included. All but one market, Kongo, yielded samples contaminated by E. coli with a total of 79 (39.5%) of 200 tested samples being positive for the organism. All (100%) E. coli isolates were of fecal origin. The mean pH was 4.41 with a range from 4.03 to 4.81.

All (100%) 50 isolates of E. coli tested were resistant to nitrofurantoin, while 16 (32.0%) were not susceptible to colistin sulphate and 17 (34.0%) were resistant to gentamycin (Table 2). Overall, the incidence of resistance to antimicrobial agents was high.

The predominant antimicrobial resistance patterns are shown in Table 3. The most common pattern was tetracycline-nitrofurantoin-sulphafurazole-kanamycin-ampicillin-co-trimoxazole (22.0%). None of the 50 isolates was sensitive to all the antimicrobial agents tested.

The frequency of production of hemolysin and heat labile (LT) enterotoxin among E. coli isolates is shown (Table 4). Of the 79 isolates, 41 (41.8%), 2 (2.5%), and 44 (55.7%) had alpha, beta, and gamma hemolytic patterns, respectively. Eight (25.8%) of the 31 E. coli strains tested produced LT enterotoxin.
DISCUSSION

That 39.5% of 200 samples of 'nono' tested contained E. coli should be of health concern when it is considered that 'nono' forms a major part of the staple food of people in northern Nigeria. Also, the concern is even more pronounced since 'nono' received minimal, if any, heat treatment. The finding is in agreement with earlier reports where 30.9% of 624 milk and milk products from various sources contained high coliform counts (23). Although the number of E. coli was not determined in the present study, an ongoing study has resulted in counts of 10^5 to 10^6 cfu/ml E. coli (unpublished data). The fairly high isolation rate found in this study could be attributed to contamination during milking, processing, and post-processing of 'nono'. Also, E. coli, being ubiquitous in nature, could contaminate milk and milk products during processing and handling (16).

Of great public health concern is the fact that all 79 isolates of E. coli were of fecal origin. This finding agrees with earlier reports (2,6) on the detection of fecal coliforms in drinking water in the environment; however, these data are not in agreement with the results of Jones et al. (16) where only 18.7% of 145 isolates of E. coli were of fecal origin. The health risk is the enteric pathogens, e.g., salmonellae, shigellae, yersiniae, etc., conceivably could be present. However, the findings in the current study are hardly a surprise since stream water is used to dilute the milk product. Although foodborne outbreaks are not reported in Nigeria, the acidic pH of 'nono', 4.03, to 4.81, may limit the multiplication or survival of pathogens at ambient temperature. Kornacki and Marth (16) reported that pH had the greatest effect on the survival of E. coli in cheese. However, being an uncontrolled fermentation product prepared under unsanitary conditions, fermentation failure may occur to allow multiplication of pathogens. 'Nono' samples had earlier been demonstrated to be contaminated by Staphylococcus aureus (28), with some of the strains being enterotoxigenic (29).

Of equal health significance was the finding that some strains (25.8%) of E. coli tested produced LT enterotoxin. This finding agrees with other reports on E. coli isolates from milk and milk products, and other foods of animal origin (25,27). In Nigeria, reports exist of instances where 50% of E. coli strains are positive for LT enterotoxin production (3).

Regardless of market source, E. coli from 'nono' samples had a fairly high degree of resistance to antimicrobial agents used. Approximately 70% or more of the isolates were resistant to all nine antimicrobial agents used except colistin sulphate and gentamycin. The general resistance observed agrees with an earlier report (10). That 90 and 88% of the isolates were resistant to tetracycline and sulphafurazole agree with reports on isolates of animal origin in Nigeria (21,22). Similarly, high resistance has been reported among food isolates of S. aureus (18) as well as those from animals (1) and human beings (1). This reflects misuse or abuse of antimicrobial agents in Nigeria.

The predominance of alpha hemolysin producers among the strains of E. coli tested agrees with reports on hemolytic isolates from various sources (4,13). Four of the eight LT enterotoxin positive strains of E. coli were alpha hemolysin producers, suggesting a relationship between enterotoxigenicity and alpha hemolysin production earlier observed by several workers (11,27).

To prevent 'nono'-borne infection by E. coli in consumers, the local fulanis should be educated on sanitary practices during milk collection from cows and processing. Pasteurization of the milk and then inoculation with a culture known to be free of contamination could also prevent infection. There is an obvious need to avoid product adulteration of this type.

| TABLE 4. Hemolytic patterns and frequency of production of heat-labile(LT) enterotoxin among E. coli isolates. |
|---------------------------------|-------------------------------|---------------------|-------------------|
| **Market**                      | **Number of isolates tested** | **Alpha** (%)       | **Beta** (%)      | **Gamma** (%)     | **Production of LT toxin** |
|---------------------------------|-------------------------------|---------------------|-------------------|
| Panladan                        | 31                            | 33.3                | 0.0               | 66.7              | 33.3                  |
| Sabon Gari                      | 12                            | 66.7                | 0.0               | 33.3              | 3                    |
| Samaru                          | 30                            | 36.7                | 3.3               | 60.0              | 0.0                  |
| Shika                           | 18                            | 22.2                | 0.0               | 77.8              | 0.0                  |
| Tudun Wada                      | 18                            | 50.0                | 0.0               | 50.0              | 3                   |
| Zaria City                      | 7                             | 28.6                | 14.3              | 57.1              | 28.6                 |
| Total                           | 79                            | 41.8                | 2.5               | 55.7              | 25.8                 |

Con't. on p. 627
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REFERENCES


Pestka et al., con't. from p. 580