Occurrence of *Escherichia coli* in smoked meat

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**ABSTRACT**

Samples of pork ‘Sa-rep’ (smoked meat) locally prepared in Mizoram and popularly consumed by the mizo people were screened, to study the prevalence of food-borne zoonotic pathogen *Escherichia coli*. Analysis of 100 samples revealed 9 (9%) isolates of *E. coli*, of these 8 belonged to 6 serogroups, viz. O2, O23, O79, O130, O139 and O172 while one turned out to be rough. The antibiotic sensitivity test showed that all isolates were resistant to erythromycin and penicillin, whereas all showed sensitivity to ciprofloxacin, gentamycin and streptomycin. Amongst the 9 isolates of *E. coli*, 8 (88.89%) turned sensitive to tetracycline. The pathogenic nature of isolates was studied by congo red dye adsorption (eRDA) test and haemolysin test wherein all isolates turned out to be positive after 36 hr of incubation in eRDA and 77.78% were found positive in haemolysin test. Occurrence of public health significant *E. coli* in ‘Sa-rep’ preparation from a place like Aizawl, wherein maximum people prefer meat consumption represents a matter of concern from public health point of view.

**Key words:** Antibiotic sensitivity, Congo red dye adsorption, Escherichia coli, Haemolysin test, Mizoram, Sa-rep, Smoked meat

*Escherichia coli* was established as a food-borne pathogen in 1971. As a human pathogen, evidence suggests that it was recognized as a cause of infant diarrhoea as early as the 1700s (Neill et al. 1994). Since the meat-borne outbreaks in the United States of this pathogen in 1982 and 1983, the status of this bacterium as a food-borne zoonotic pathogen is unquestioned (Jay 2000). The pathogen has often been found to be associated with a variety of human as well as animal ailments, including gastroenteritis, urinary tract infections, wound infections and septicemia (Sojka 1971, Senior et al. 1992, Kapur et al. 1992).

The organism also has a value as safety indicator as recommended by the International commission on the Microbiological Specifications for Food (ICMSF) (Jay 2000) including meat and meat products.

In Mizoram majority of population is meat eater. As per the data of 2003-04 census 87,121 animals were slaughtered in the state which included 64,450 (75%) pigs, and 50,397 (77%) were in the Aizawl district alone (Sangghina 2005).

‘Sa-rep’ which meant smoked meat (Sa-is meat, and rep­means dehydrated or dry in Mizo language) is preferred by majority of the mizo people. Considering the local butchering practices, liking of the mizo community for the meat and meat products and importance of the *E. coli* pathogen as an indicator organism in food and meat industry as well as its zoonotic implications as food-borne pathogen, the present work was envisaged, which seems to be the first systematic study conducted in Mizoram so as to get the picture of the prevalence of *E. coli* in Mizoram so as to get the picture of the prevalence of *E. coli* in popularly consumed ‘Sa-rep’ along with virulent isolate, if any, and their in *vitro* drug sensitivity profile against some common antibacterial agents.

**MATERIALS AND METHODS**

Pork ‘Sa-rep’ samples (100) each weighing approximately 50g were collected aseptically in the sterile sachets from various shops/butcher shops. The collected samples were immediately transported to the laboratory for further isolation studies.

**Bacteriological examination of the samples:** Each sample of ‘Sa-rep’ was aseptically transferred to the sterile beaker and added with sufficient quantity (approximately 450 ml) of chilled 0.1% peptone water. Then the sample was homogenized at 6 000 rpm for 2 min to make it complete homogenate. One ml of the homogenate was then aseptically transferred into sterile petri-dish and eosiline methylene blue (EMB) agar was poured. The inoculated petri-dishes were allowed to solidify and then incubated at 37°C for 24 h. After incubation the colonies showing typical metallic sheen were picked up and inoculated onto nutrient agar slants and bovine
heart infusion (BHI) broth for further morphological and biochemical characterization as per the methods of Buchanan and Gibbon (1994).

Serotyping: The isolates, after their morphological and biochemical characterization, identified as *E. coli*, were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh, 173204 for serogrouping.

Antibiotic sensitivity test (AST): The susceptibility of the *E. coli* isolates to the antimicrobial agents was determined by using *in vitro* single disc diffusion method (Bauer et al. 1966). The antimicrobial drug discs used for the test were erythromycin, gentamycin, streptomycin, penicillin, tetracycline and ciprofloxacin. The test was read by comparing the inhibition zone with the standard chart provided by the manufacturer.

Congo red dye binding assay/Congo red dye adsorption assay (CRDA): The test was performed as per Iahiguro et al. (1985). Briefly, the *E. coli* isolates were streaked onto triplicate soy agar containing 0.05% congo red dye. The inoculated plates were then incubated up to 36 hr at 37°C. The colonies were observed after 18 and 36 hr of incubation. Appearance of brick red coloured colonies indicated adsorption of dye by the isolate and accordingly determined as congo red positive, whereas, the white colonies were taken as congo red negative strain.

Haemolysin test: The production of haemolysin by the isolates was checked as per Brenden and Janda (1987) with some modification. For this all the isolates of *E. coli* were streaked onto blood agar (blood agar base added with 5% defibrinated rabbit blood), the inoculated plates were then incubated at 37°C for 24 hr. The production of haemolysin was indicated by a clear halo zone surrounding the colony.

RESULTS AND DISCUSSION

Bacteriological isolation studies on 100 pork 'Sa-rep' (smoked meat) samples revealed 9 isolates of *E. coli*, thus, prevalence of 9% could be noted. These isolates belonged to 6 serogroups as 02, 023, 079, 0130, 0139 and 0172. Among the 9 isolates, 2 each belonged to serotypes 02 and 079, whereas, 1 each was serogrouped in the remaining serogroups and 1 turned out to be rough strain (Table 1). These results are in partial accordance with studies of isolation attempted from animals and birds by Yadav et al. (2005) where they have reported serotypes 023 and 0139 from mutton samples, Sikdar et al. (1994) for serotype O2 and rough strain from cases of piglet diarrhoea, and Sharda et al. (1999) for serotype O79 from pathogenic cases of poultry.

The resistance of all *E. coli* isolates to erythromycin and penicillin observed in the present study is in agreement with the findings of Wani et al. (2004). They have reported cent per cent isolates resistant to these antibiotics. This may be attributed to the frequent use of these drugs in treatment of pigs in Mizoram. The sensitivity of all *E. coli* isolates to gentamycin is totally in agreement with observations of Wani et al. (2004) and Miles et al. (2006) where sensitivity by cent percent isolates has been noted. The results are also comparable with those by Sikdar et al. (1994), Borah and Das (1996) and Seh et al. (2009) where, sensitivity to gentamycin was reported in 81.65%, 94.49% and 90.90% *E. coli* isolates, respectively. Observation that 100% isolates were highly sensitive to third generation antibiotics like ciprofloxacin is in agreement with Pattainik et al. (1996) and Mukhopadhayay et al. (1998). Sensitivity of 88.89% *E. coli* isolates to tetracycline is in accordance with observations of Vidovic and Korber (2006), where, 97% *E. coli* isolates were reported to be sensitive to the antibiotic. The sensitivity to streptomycin is in partial accordance with reports of Borah and Das (1996) where, 21.26% isolates were sensitive for the antibiotic. However, sensitivity to streptomycin and tetracycline respectively by 100% and 88.89% of *E. coli* isolates in the present study are contrary to the reporters of Mukhopadhayay et al. (1998) and Wani et al. (2004) who reported 100% resistance against both antibiotics. This may

<p>| Table 1. In-vitro antibiotic sensitivity pattern of <em>E. coli</em> isolates from 'Sa-rep' (smoked meat) |
|-------------------------------------------------|----------|----------|----------|----------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Serogroup</th>
<th>E</th>
<th>G</th>
<th>T</th>
<th>P</th>
<th>S</th>
<th>Cf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa-3</td>
<td>079</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sa-7</td>
<td>0139</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
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<td>0130</td>
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<td>+</td>
<td>-</td>
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<td>Rough</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sa-15</td>
<td>023</td>
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<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>Sa-21</td>
<td>0172</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
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<td>+</td>
</tr>
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<td>-</td>
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<tr>
<td>Sa-88</td>
<td>02</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

E, erythromycin; G, gentamycin; T, tetracycline; P, penicillin; S, streptomycin; Cf, ciprofloxacin
be attributed to the fact that the good climatic conditions and the stall fed practice along with the individual rearing of animal (as the high terrains do not permit to rear them together in groups) by the mizo farmers does not necessitate the frequent use of these antibiotics in animal husbandry in Mizoram.

Congo red dye binding assay/Congo red dye adsorption assay (CRDA) of all 9 E. coli isolates revealed that 6 (66.67%) isolates positive for CRDA after 18h of incubation; however, after 36h of incubation; all turned positive showing brick red coloured colonies (Table 2). The results of CRDA are in agreement with observations of Malik et al. (2000) where the E. coli isolates negative for CRDA after 18h were reported to show positivity after 36h of incubation. The serogroups which showed positivity for CRDA after 18h or O79, O130, O2, O79 and O2 (Table 2). The ability of E. coli isolates to bind congo red dye and produce pigmented colonies on defined media can be used to differentiate avirulent and virulent strains (Berkhoff and Vinal 1986, Sharda et al. 1999), and the property to bind congo red dye is used as predictor of virulence and as a marker for pathogenic strains of E. coli (Singh and Gupta 1996). In the present study it is noted that among the serogroups showing CRDA positivity, O2 and O79 were reported from piglet diarrhoea (Sildar et al. 1994) and poultry (Sharda et al. 1999) respectively. The CRDA positive O23 and O139 serotypes were reported from mutton (Yadav et al. 2005).

Among the 9 E. coli isolates, 7 (77.78%) turned out to be haemolytic on rabbit blood agar (Table 2). This is in partial accordance with Dubey et al. (2000) who reported that 30.4% E. coli isolates were positive for haemolysin test on ox blood agar. However, Wani et al. (2004) and Sharda et al. (1999) reported haemolytic positivity among 2.63% and 0% E. coli isolates, respectively, on sheep blood agar. The variation may be due to the pathogenic nature of the isolate itself or the possibility if it is due to variable source of blood has to be ruled out. Thus, in the present study a good correlation among the in vitro pathogenicity tests (CRDA and haemolysin test) was found, as except 2, all other 7 CRDA positive E. coli isolates showed haemolytic activity (Table 2).

In the present investigation the prevalence of zoonotically important pathogen E. coli among the smoked meat samples with CARD and haemolytic positivity is a matter of concern in the meat industry from public health point of view especially in state like Mizoram where meat consumers are more.

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REFERENCES


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OCCURRENCE OF ESCHERICHIA COLI IN SMOKED MEAT


