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Molecular Characterization of *Escherichia coli* from Pork Meat by Whole Cell Protein Finger Printing

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Foodborne infections are major concerns for public health, and are occurring due to microbial contamination of meat. E. coli blotches on surfaces of the meat especially pork and beef. Meat, as a source of protein, and provides energy to our body pork meat has inhabited as special place in the diet of majority of Thai-Population for a wide variety of reasons such as tradition and availability. This study was aimed to evaluate the protein patterns among E. *coli* strains from pork meat samples of thalath Ongkharak market, Nakhonnayok, Thailand. To evaluate the similarity or intra-specific polymorphism degrees based on whole-cell protein fingerprinting, plasmid profiles and antibiotic resistance patterns. It was determined that the SDS-PAGE method may provide better criteria than plasmid and antimicrobial susceptibility for the taxonomic and epidemiological studies of *E. coli* isolates. Molecular characterization of isolates of E. coli was carried out by whole cell protein analysis by SDS-PAGE. Whole cell protein analysis of isolates carried out by SDS-PAGE, and were observed visually and compared among samples.

Keywords: Pork Meat, *E. coli,* Prevalence, Chloramphenicol, protein, and fingerprinting.

Globally, foodborne pathogens are the origin of diseases and deaths. To prevent the foodborne pathogens billions of dollars spending[1]. *E. coli*, is one of the common origin for foodborne diseases in mammals. Resistant strains of *E. coli* infect at all the age groups and acid resistance, wide range of infections are the severe consequences[2]. Severity of the infections depends on the host susceptibility, virulence of the *E. coli*, and dose. Prevalence of resistant strains of *E. coli* results mild and severe bloody diarrhea, hemorrhagic colitis, and or hemolytic uremic syndrome, which lead to kidney failure[2, 3]. Livestock animals are the primary reservoirs of *E. coli* and meat products are determined ass major root cause of foodborne transmission[2,4]. Carcass contamination occurs through skin-to-carcass or fecal-to-carcass transfer of the pathogen during slaughter process at processing plants[5,6,7]. These are the major concerns for human infection. However, during the processing of meat direct, indirect and or cross-contamination may occur. Antimicrobials are using mainly reduce pathogen shedding[8,9] and washing of skin and carcass[10].

For the first time, by using genetic fingerprinting techniques, Kudva *et al*.[11] found multiple strains of *E. coli* O157:H7 in a single flock of sheep and showed that a single animal shed multiple strains simultaneously and that strains shed by individuals changed over time. *Escherichia coli* O157:H7 has been isolated from animal drinking water, animal feed, flies, and a

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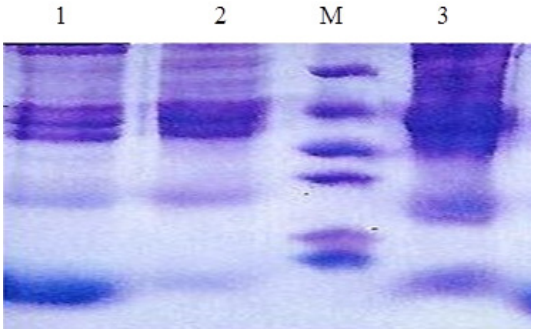
pigeon at dairy farms in Wisconsin[12]. The majority of isolates collected at these farms had the same genetic fingerprint. Although various methods are available for genetic characterization of bacterial isolates, random amplification of polymorphic DNA (RAPD) has been used successfully in the past for *E. coli* O157:H7[13, 14,15] and is less costly and time-consuming than other methods. As a source of animal protein, goat meat has for long occupied a special place in the diet for a variety of reasons including taste preference, prestige, religion, tradition and availability, in almost all the communities of the country with the nutritional aspects being included more recently. Meat was the first important food that met up the hunger of ancient people living in cave[16]. It plays a very vital role in keeping the human body strong in order to provide energy and health[17]. But, microorganisms present in meat may be harmful for human and may cause spoilage and may be used as indicator organisms. Many researchers have isolated and identified heterogeneous types of microflora from fresh meat.



Whole-cell protein profiles of meat E. *coli*
Lane: 1: SDS-PAGE protein profiles of *E. coli* isolates from fresh pork meat sample.
Lane: 2: SDS-PAGE protein profiles of *E. coli* isolates from frozen pork meat sample.
Lane: 3: SDS-PAGE protein profiles of *E. coli* isolates from pork meat sample.
Lane: M: 1Kb DNA marker

Fig. 1. Extraction of whole cell protein of E. coli resistant strains by PAGE

Protein profiling by SDS-PAGE is a reliable and reproducible molecular technique that has been used by many workers to type various microorganisms of epidemiological interest. This technique was utilized for differentiating the pathogenic and the non-pathogenic strains. Plasmid analysis has also proved a useful method for differentiating bacterial isolates[18, 19]. The number and size of the plasmids present is used as the basis for strain identification. This strain typing technique has been used successfully for analysis of outbreaks of nosocomial infections[20] and community-acquired infections[21] caused by a variety of species of Gram negative rods. In the present study aimed to investigate the whole cell protein patterns for characterizing and differentiating *E. coli* isolates from meat samples.

MATERIALS AND METHODS

A total number of 74 pork meat samples were collected equally from slaughter yards and meat stalls. After collection, bacteriological analysis of the samples was performed to asses the selected microbial attributes in goat meat cuts of different sources by using MacConkey (MC) agar or nutrient agar medium to find out the sanitary quality and identification of bacterial strains in goat meat. Bacteria were cultured on these media in aerobic conditions at 37°Cfor 24 h[22] and colonies were identified by standard biochemical tests.

Whole cell protein analysis

The total protein samples were extracted as described by the Kishore *et al*.[23] (1996). Total protein analysis was carried out by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described previously. Each run

Table 1. Prevalence of antimicrobial resistance in E. coli isolates of pork meat

Meat sample	Tetracycline(%)	Ampicillin (%)	Chloramphenicol (%)	Streptomycin (%)
Pork (n=46)				
Sensitive	0	12(26.08)	11(23.91)	15(32.61)
Resistance	28(60.9)	21(45.66)	15(32.61)	18(39.1)
Intermediate	18(39.1)	13(28.26)	20(43.47)	13(28.26)

included marker proteins of known molecular weight (Fermentas). The gel was stained overnight with Coomassie Brilliant Blue G-250 according to Bushuk[24] and Demiralp[25].

RESULTS AND DISCUSSION

Out of 74 samples 46(60.3%) were detected as positive by disc diffusion method, 60.9% of isolates were resistant to tetracycline, ampicillin (45.66%), chloramphenicol (32.61%) and streptomycin (39.1%) respectively. However, 39.1%, 28.26%, 28.26% for tetracycline, ampicillin, chloramphenicol and streptomycin respectively. notwithstanding, 26.08 %, 23.91%, 32.61% were sensitive to tetracycline, ampicillin, chloramphenicol and streptomycin respectively (table.1). The highest prevalence of antimicrobial resistant strains in the study area shows the high concern for the community health. Environmental sampling would be helpful in this regard, to check whether it has any impact on the persistence and dissemination of resistant strains. High prevalence of resistant strains indicating the detrimental and devastating effects of excess usage of antimicrobials.

Whole-cell protein profiles of meat E. *coli* isolates obtained by SDS-PAGE were inspected visually and compared among samples. The protein profiles of all *E. coli* isolates exhibited different banding patterns. The genetic distance of the strains based on whole protein profiles of *E. coli* isolates was detected. SDS-PAGE is used in studies to discriminate the bacterial strains. Plasmid analysis is also used method for differentiation among some bacterial strains[26, 27]. This study showed that antibiotic susceptibility, plasmid DNA and SDS-PAGE analysis of whole cell proteins have a discriminatory power to distinguish the *E. coli* strain. *E. coli* strains are routinely exposed to a wide range of antimicrobial agents. *E. coli* also has a very wide natural distribution[28,29] and a propensity for plasmid carriage[29]. Resistance to various antibiotics is relatively common in clinical pathogens in Turkey and also common in *E. coli* strains[30,31,32] and it is frequently plasmid-mediated[33].

The high prevalence of resistance to the various class of antimicrobials in the clinical and animal origin indicates further investigations to confirm. However, it is impossible to mimic epidemiologic association between the observed high resistance to the antimicrobials. This may depend on frequency of utilization of these antimicrobials in the study area, and it demonstrate that antimicrobials could be the most commonly used in Thailand to treat human and livestock animals. Hence, need further studies to elucidate the devastating effects of antimicrobials.

Resistance in *E. coli* was observed at high prevalence from the pork samples, collected from raw meat sellers. It indicates transmission of the pathogen by the animal to human. This investigation indicates that the high prevalence of *E. coli* in pork meat could be due to current sanitary systems at retail shops and processing units. Further epidemiological investigations needed to initiate on pork production and processing continuum are recommended to further substantiate the findings of the study.

Plasmids were screened to determine their antibiotic resistance profiles. In this study, plasmids were screened to determine their antibiotic resistance profiles. It was observed that there was no close relation between plasmid occurrence and multiple antimicrobial resistance in isolates. This could be due to isolates has resistance without plasmids. SDS-PAGE analysis was the most efficient method for characterizing *E. coli* species used in this study, because these species showed differences in their electrophoretic protein patterns.

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