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Research Article

A Study of Antibiotic Resistance, Biochemical and Molecular Characterization of *E.coli* Strains Isolated from Meat Samples

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ABSTRACT

A Total of 18 meat samples were collected from different slaughter houses and meat stalls in and around Tirupati, India. Samples were cultured on MacConkey, Nutrient agar medium and 9 E.coli isolates were obtained. The isolates were confirmed by gram staining and biochemical tests. These 9 isolates were tested for antibiotic susceptibility, highest rate of resistance were observed against ampicillin and most of the E.coli isolates showed resistance to two or more antibiotics and where therefore multi drug resistance. Molecular characterization of E.coli strains were carried out by plasmid analysis and whole cell protein analysis by SDS-PAGE. Plasmid isolation was done by alkaline lysis method and plasmid bands were observed in 7 isolates. Whole cell protein analysis of E.coli isolates obtained by SDS-PAGE were inspected visually and compared with each other. The protein profiles of all isolates exhibited different banding pattern.

Keywords: *E.coli*, Antibiotic Susceptibility, Plasmid analysis, SDS-PAGE

INTRODUCTION

Food-borne infections leading from microbial contamination of meat from slaughter house and meat stalls continue to be a health concern for the public at large. As a source of animal protein, goat meat has occupied a special place in the diet for a variety of reasons including taste preference, prestige, religion, tradition and availability. Meat was the first important food that met up the hunger of ancient people living in cave[1]. It plays a very vital role in keeping the human body strong in order to provide energy,

health and vigor[2]. 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 micro flora from fresh meat.

Escherichia coli is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Pathogenic *E. coli* has been recognized as a major cause of neonatal septicemia, diarrhea in children, neonatal meningitis and urinary tract infection in human beings. Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. At present, *E. coli* has become a great concern in both human and veterinary practices, because of the indiscriminate use of antimicrobial agents, tracing to the emergence of antibiotic resistant strains[3,4]. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms.

More recently the importance of Gram-negative bacteria has increased since the advent of broad-spectrum antibiotics because these organisms often carry multiple antibiotics resistance[5]. Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more common. These beta-lactamase enzymes make many, if not all, of the penicillin's and cephalosporin's ineffective as therapy. Extended-spectrum beta-lactamase producing *E. coli* are highly resistant to an array of antibiotics and infections by these strains.

In microbiological analysis associated with the epidemiological investigation of eruptions, it is often necessary to obtain more detailed identification and characterization of the organisms involved than can be provided by conventional methods[6]. Microorganism Strain typing can be classified into phenotypic and genotypic methods. Conventional methods such as morphological, biochemical and physiological tests, antibiogram analysis are often based on phenotypic characteristics. These methods can be time consuming, labor intensive, rely on specific media, multiplication of the target organism and do not use genetic information, which can be used to discriminate almost closely related organisms. Most of these techniques are not sufficiently sensitive to distinguish different strains and they are affected by physiological factors. Alternative approaches such as molecular characterization of microorganisms are frequently used by physicians, microbiologists, and epidemiologists to provide evidence of genetic relatedness as an aid in the epidemiological investigation of infectious diseases.

The application of molecular analyses such as a whole cell protein analysis and plasmid analysis to investigations of infectious disease outbreaks has resulted with the provider of many useful markers that distinguish the epidemic clone of a particular pathogen and helped the identification of specific vehicles of infection. Strain typing technique has been used successfully for analysis of outbreaks of nosocomial infections[7] and community-acquired infections[8-10] caused by a variety of species of Gram-negative rods. Whole cell protein profiles by SDS-PAGE are an authentic and consistent molecular technique that has been utilized for differentiating between pathogenic and the non-pathogenic strains. Plasmid analysis has also proved a useful method for differentiating bacterial isolates [11, 12]. The number and size of the plasmids present is used as the basis for strain identification.

The purpose of present study is to evaluate antibiotic susceptibility, biochemical and molecular characterization of *E.coli* strains isolated from meat samples. As meat is the major source of infection spreading from animals to human, collection at slaughter house was identified for this study. In view of the microbial implication in handling, slaughtering, dressing, processing and distribution of meat and meat products which may endanger human health, the study was undertaken to determine the extent of microbial contamination of goat meat from slaughter yards and from meat stalls at late market hours.

EXPERIMENTATION

Sample collection and isolation of *E.coli* isolates: A total of 18 meat samples were aseptically collected in a container equally from three slaughter yards and meat stalls in and around Tirupati, Andhra Pradesh, India. Meat samples were brought to laboratory within 30-45 min using ice box. Samples include- fresh meat, intestine, frozen meat of goat.

Meat samples were gently homogenized, serially diluted and spread on MacConkey agar and Nutrient agar medium followed by incubation at 37°c for 24hours. The isolated bacterial colonies were purified to homogeneity by quadrant streaking.

Identification of *E.coli* strains: Identification of *E.coli* strains were carried out by examining the isolates according to their cultural, morphological and biochemical characterization. The *E.coli* isolates were identified based on the biochemical tests outlined in the Bergey's manual of determinative bacteriology.

Antibiotic susceptibility/resistance screening: The sensitivity/resistance of the *E.coli* strains to four antibiotics such as Ampicillin, Tetracycline, Chloramphenicol, and Streptomycin were tested by Kirby-Bauer disc diffusion technique. Nutrient agar plates were inoculated with *E.coli* isolates. Antibiotic disc were placed on the surface of the agar plates and incubated at 37°c for 24h. After incubation, the zone size were classified as Resistant [R], Intermediate [I], Sensitive [S], according to the zone table consistituted by Clinical and Laboratory Standard Institute [CLSI].

MOLECULAR CHARACTERIZATION

Plasmid DNA Analysis: Plasmid DNA was isolated according to the method of Maniatis.et.al.[13] by alkaline lysis method with SDS. Plasmid DNA was subjected to qualitative and quantitative analysis by using UV-VIS spectrophotometer at 260nm and 280nm. Plasmid DNA was purified and subjected to agarose gel electrophoresis. Electrophoresis was carried out on 0.8% agarose gel in TAE buffer for 4h at 100V. The gel was observed under gel doc. Standard DNA molecular weight markers were used to estimate the plasmid size.

Whole Cell Protein Analysis: The total protein samples were extracted as described by Kishore.et.al[14]. Total protein analysis was carried out using SDS-PAGE as described in Laemmli[15]. The gel was stained overnight with Coomassie Brillant blue G-250 and distained according to Bushuk.et.al.[16], Demiralp.et.al.[17] and analyzed.

RESULTS AND DISCUSSION

The microbiological investigation was conducted to determine the level of contamination of meat processed by butchers in slaughter yards and meat stalls. A total number of 18 meat samples were collected equally from slaughter yards and meat stalls. After collection, bacteriological analysis of the samples were performed to assess the selected microbial attributes in meat cuts of different sources by using MacConkey (MC) agar and nutrient agar medium to find out the sanitary quality and identification of bacterial strain in meat. Two *E.coli* isolates from fresh meat, four from intestine sample and three from frozen meat were isolated and confirmed by staining and biochemical characterization **(Table 1& Fig.1).**

Out of the total, 4 antibiotics used only four E. coli isolates i.e., strain 1 from fresh meat, strain 3 from intestine sample and strains 7 and 8 from frozen meat showed resistance to all four antibiotics **(Table-2).** It has been reported that pathogenic isolates of *E.coli* have relativity high potential for developing resistance. Analysis of the susceptibility testing of *E. coli* strains isolated from meat has demonstrated that the rate of resistance to ampicillin is highest among all the antimicrobials. Most of the strains show resistance to more than two drugs and hence multidrug resistance.

Table -1: Cultural, Morphological and Biochemical Characteristics

Phenotypical and biochemical Characterization	*E.coli* Isolates
Colony Morphology	Small, Smooth, Circular, Entire, Creamy white, Slightly Raised, Rod shaped
Gram Staining	Gram Negative
Motility	Motile
Indole test	Positive
Methyl Red test	Positive
Voges Proskauer	Negative
Citrate test	Negative
Catalase test	Positive
Starch hydrolysis	Positive
Casein hydrolysis	Positive
Oxidase test	Negative
Urease test	Negative

CITRATE TEST-
(NEGATIVE)

INDOLE TEST-
(POSITIVE)

METHYL RED TEST-
(POSITIVE)

CATALASE-
(POSITIVE)

Fig.1: Biochemical test

Table-2: Antibiotic susceptibility of isolated *E.coli* strains.

Antibiotic	Strain 1	Strain 3	Strain 7	Strain 8
Ampicillin	R (+ +)	R (+ +)	R (+ +)	R (+ +)
Tetracycline	R	R	R	R
Chloramphenicol	R	R	R	R
Streptomycin	R	R	R	R

R: Resistant, ++: More Resistant

Besides, amongst the enteric pathogens, resistance of E. coli was observed to be increasing, especially to first line, broad spectrum antibiotic, such as ampicillin and others. In the present investigation, high resistance of *E.coli* to numerous antimicrobial agents (antibiotics) was observed. Such high number of resistance by E. coli, these results are contradictory to those reported by *M. Boten, et.al* [18]. The situation indicates a threat and a possibility that the *E. coli* could have become resistance to many more antibiotics to which it showed susceptibility earlier.

In view of these results, the studies on *E. coli*, focusing on the changes on molecular level, could provide valuable insights for its management. Plasmid profiling demonstrated that 7 isolates contain plasmid DNA. Most of the isolates have 1 to 3 plasmid bands. The most common plasmid of 1000bp, 6000bp was detected in almost all strains isolated.

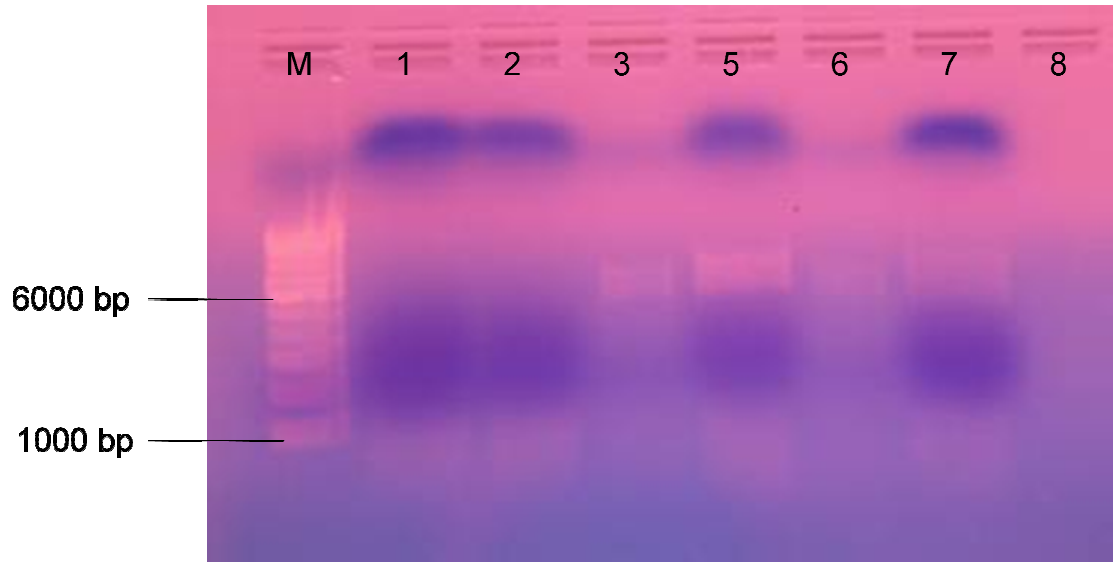

Fig.1: Plasmid Profile of *E.coli* isolates: M=1-10 Kb Marker, Lane 1, 2, 3, 5, 6, 7, 8= *E.coli* strains.

This drug resistance increases as a function of time and microorganisms exposure to many factors like antibiotics, chemicals, etc. Besides, the bacteria acquire resistance through different routes, such as natural or intrinsic resistance (inaccessibility of the target, multidrug efflux systems and drug inactivation), mutational resistance (drug target site modification, reduced permeability or uptake, metabolic by pass and derepression of multidrug efflux), extra chromosomal or acquired resistance (drug target site

modification, reduced permeability or uptake, metabolic by pass and derepression of multidrug efflux). All these mechanisms of antibiotic resistance warrant a detailed investigation of multiple factors, with prioritization of the studies of molecular characterization. Multi drug resistance has serious implications for the empiric therapy of infections caused by *E.coli* and for the possible co-selection of antimicrobial resistance mediated by multi drug resistance plasmids[19]. Thus, the studies confirm the important role of plasmid numbers and plasmid size that controls the resistance characteristics in *E. coli*.

The analysis of cell protein extracts of the selected strains of *E.coli* isolated from meat samples by SDS-PAGE showed similar and different profiles.

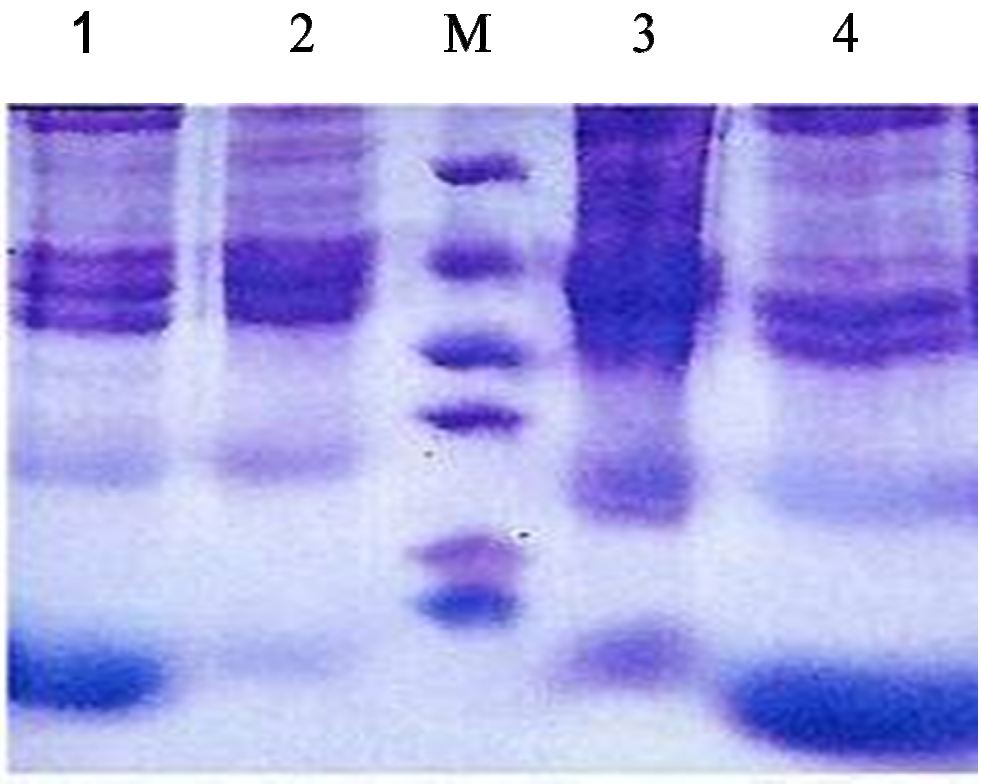

Fig.2: SDS-PAGE Whole cell protein profiles of *E. coli* isolates. M-molecular weight standards (KDa) (116-β-galaktosidase, 66- Bovine serum albumin, 45- ovalbumin, 35- lactate dehydrogenase, 25- restriction endonuclease *Bsp*98, 14-lysozyme). Lane-1: *E.coli* 3, Lane-2: *E.coli* 4, Lane-3: *E.coli* 6, Lane-4: *E.coli* 7.

The protein bands are common in almost all the examined strains, while other minor differences in the protein pattern may be due to the nature of the meat and environmental factors involved in the isolation of these strains. These results suggest that each species yielded a different electrophoretic pattern. However, a slight variation among the examined strains may be due to different growth conditions and similarities in the band pattern shows that they are similar in the activity. It is furthermore added that these slight dissimilarities may be due to the difference in the origin of the strains.

SUMMARY AND CONCLUSION

In this study, we tried to distinguish *E. coli* strain using plasmid profiles, SDS-PAGE and antibiotics susceptibility. In order to improve control and prevention strategies against infectious diseases, microbial pathogens need to be identified quickly and accurately. Quality of meat processed in meat stall is very low

due to improper handling. Microorganisms can be identified both phenotypically and genotypically. In clinical settings, molecular techniques provide more sensitive, faster, and easier tools than conventional microbiological methods of diagnosis.

Antimicrobial resistance plasmids have been increasingly associated with both Gram-positive and Gram-negative bacterial infections. This trend is accelerated by the fact that *E. coli* is a common enteric commensal of mammals and a common cause of human infections. As such, *E. coli* strains are routinely exposed to a wide range of antimicrobial agents. *E. coli* also has a very wide natural distribution and a propensity for plasmid carriage. Resistance to various antibiotics is relatively common in *E. coli* strains and it is frequently plasmid-mediated. In this study, plasmids were screened to determine their antibiotic resistance profiles. It was observed that there is not a close relation between plasmid occurrence and multiple antibiotics resistance for all of the isolates because some of the isolates, with plasmid has no antibiotic resistance.

SDS-PAGE analyses were the most efficient method for characterizing *E.coli* species used in this study, because these species showed differences in their electrophoretic protein patterns. To differentiate *E. coli* strains present in food infections, more than one method should be used, since care in handling of these strains is very important factor in accuracy of research involving clinical, epidemiological and taxonomic studies. 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.

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