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Original Research Article

Isolation and Characterization of Microorganisms from Raw Meat Obtained from Different Market Places in and Around Chennai

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ABSTRACT

Foods, by their nature are nutritious and easily metabolisable and therefore offer suitable substrates for the growth and metabolism of microorganisms. Food borne disease is a pervasive problem caused by consumption of contaminated food and water. The microorganisms that ultimately bring about the spoilage of flesh foods are either present at the time of slaughter, or introduced by workmen and their cutting tools, or by water and air in the dressing, cooling and cutting rooms. Meat is an ideal medium for many organisms to grow because it is high in moisture, rich in nitrogenous compounds (e.g. amino acids, peptides, and proteins) and plentifully supplied with minerals and accessory growth factors. Therefore in this study, an attempt has been made to study the microbial contaminants of meat sold in market places in and around Chennai. The bacterial and fungal contaminants were isolated and identified using specific culture techniques. The predominant bacterial pathogen isolated was *Escherichia coli, Salmonella spp., Staphylococcus aureus* and *Pseudomonas.* The predominant mold pathogen isolated was *Penicillium spp.*, followed by *Mucor spp*., *Aspergillus niger*, *Alternaria spp., Sporotrichum*, *Aspergillus fumigates*. The predominant yeast pathogen isolated was *Trichosporon spp.*, followed by *Rhodotorula spp.*, *Candida spp*. Thus the present study reveals the fact that raw meat is heavily contaminated with the high incidence of bacterial pathogen and low incidence of fungal pathogens. Therefore there is an urgent necessity to minimise the contamination of meat sold in market places by proper sanitation and inspection practices.

Keyword: Food borne disease; meat; microbial pathogens

INTRODUCTION

Food-borne disease is any illness that results from eating contamined food or beverages and is also frequently referred to as food poisoning.

It is caused by pathogenic bacteria, viruses, parasites that contaminate food, as well as chemical or other natural toxins derived from poisonous mushrooms. Food– borne diseases

are a major public health problem leading to high morbidity and mortality worldwide. The global burden of infectious diarrhoea involves 3-5 billion cases and nearly 1.8 million deaths annually, mainly in young children. [1]

Meat is animal flesh derived from the mammalian species that is used as food for human consumption. Meat refers to the skeletal muscles and associated fat, but it may also describe other edible tissues such as organs, livers, skin, brains, bone marrow, kidneys or lungs. Meat is generally eaten cooked, but there are many traditional recipes where meat is eaten raw or is partially cooked. In recent years, the health benefits of meat as a regular part of the human diet may be offset by risks.

Meat, like any food, can also transmit certain diseases, but complete cooking and avoiding recontamination reduces this possibility. Undercooked pork sometimes contains the parasites that cause trichinosis or cysticercosis. Chicken is often contaminated with *Salmonella enterica* which is a disease-causing bacteria. Minced beef can be contaminated during slaughter with disease-causing *Escherichia coli* O157:H7 originating from the intestinal tract or hide if proper precautions (such as steam pasteurization or organic acid treatment) are not taken. [2].

It has been pointed out that during slaughter, dressing, and cutting, microorganisms come chiefly from the exterior of the animal and its intestinal tract but more are added from knives, cloths, air, workers, carts, boxes, and equipment in general. A great variety of kinds of organisms are added, and so it can be assumed that under ordinary conditions most kinds of potential spoilage organisms are present and will be able to grow if favourable conditions present themselves [3]. The present study is an attempt to study the microbial contaminants of meat sold in market places in and around Chennai city.

MATERIALS AND METHODS

Sample Collection:

A total of 20 samples each of raw poultry meat and raw mutton meat were collected from four different regions of Chennai. About 5 samples were collected from different retail outlets in each different region. About 100 grams of meat samples were collected in clean, dry and sterile polythene bags and transported to the laboratory for microbiological analysis within one hour or refrigerated at 4°C till further analysis were carried out and processed no later than 96 hours after purchase [4].

Processing of samples

The samples were aseptically cut into thin smaller pieces using sterile knife. The analytical portions were placed in separate sterile plastic bags to which 250 ml of buffered peptone water was added. The bags were shaken vigorously and the sample rinsate was collected.

Direct Plating for Culture

For cultivation of *Escherichia coli*, the sample rinsate was inoculated into double strength MacConkey Broth at 37°C for 24 hours after which it was plated onto Eosin Methylene Blue agar. For the isolation of *Salmonella spps.,* the sample rinsate was inoculated into double strength lactose broth at 37°C for 24 hours after which it was plated onto Salmonella and Shigella agar. For identification of *Vibrio spps.*, the rinsate was inoculated into alkaline peptone water at 37°C for 24 hours after which it was plated onto Thiosulphate Citrate Bile salt sucrose agar. For the identification of *Staphylococcus aureus* and other non-fastidious bacteria, the rinsate was inoculated into nutrient broth at 37°C for 24 hours after which it was plated onto Mannitol Salt agar. For the isolation of moulds and yeast, the rinsate was inoculated onto Sabouraud's Dextrose agar and incubated at 25°C and 37°C respectively [5].

Microscopy and Colony morphology Identification

Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopically) for colonial appearance, size, elevation, form, edge, consistency, colour, odour, opacity, haemolysis and pigmentation and the results was recorded. Gram's staining from the colonies provided a preliminary identification of the pathogenic bacteria. Fungal moulds and yeasts were identified by performing Lactophenol cotton blue staining [6].

Biochemical Characterisation

Biochemical characterisation of the bacteria was done by performing specific tests such as catalase, oxidase, TSI, Indole,Methyl red, Voges Proskauer and citrate tests, carbohydrate fermentation tests, nitrate tests, growth at 42°C, coagulase, DNAase tests, mannitol fermentation, O/F tests and sensitivity to Novobiocin, bacitracin and furazolidone. Identification of yeast was done using Corn meal agar morphology [7].

Antibiotic Sensitivity testing for the isolates

Antibiotic sensitivity testing by the Kirby Bauer's disc diffusion method was performed for the isolates using commercially available antibiotic discs on Muller – Hinton agar (MHA). Standard suspensions of the isolates were adjusted to 0.5 McFarland Standard. Immediately after standardization a sterile cotton swab was immersed into bacterial suspension and a lawn culture was performed on the surface of MHA plate. Commercially available antibiotic discs were arranged on the surface of inoculated plates. The plates were incubated at 37^0C for 16-18 hours. The antibiotics were selected as per NCCLS guidelines. After incubation the zone diameter was measured for each antimicrobial agent and it was compared with NCCLS chart. Thereby the zone of inhibition was interpreted as sensitivity (S), Intermediate (I) or resistant (R). [8]

RESULTS

A total number of 40 meat samples (Chicken - 20, Mutton-20) were examined for the presence of bacterial and fungal pathogens. The 40 meat samples were collected from different market places in and around Chennai. The first five samples (of chicken and mutton) were collected from Pallavaram (West Chennai), the second five samples (of Chicken and mutton) were collected from Velachery (South Chennai), the third five samples (of Chicken and mutton) were collected from Tondairpet (North Chennai), and the last five samples (of Chicken and mutton) were collected from Santhome (East Chennai). The predominant bacterial pathogen isolated was *Escherichia coli* (70%) followed by *Staphylococcus aureus* (25%), *Salmonella paratyphi* B (20%), *Salmonella paratyphi* A (15%), *Salmonella typhimurium* (15%), *Pseudomonas* (10%) and *Shigella flexneri* (2.5%) as shown in (Table 1)

The predominant mold pathogen isolated was *Pencillium spp.* (10%) followed by *Mucor spp* (7.5%), *Aspergillus niger* (5%) *Alternaria spp* (5%) *Sporotrichum* (2.5%) *Aspergillus fumigatus* (2.5%) as shown in (Table 2). The predominant yeast pathogen isolated was *Trichosporon spp.* (12.5%) followed by *Rhodotorula spp.* (10%), Candida *spp.* (5%). The chicken samples were heavily contaminated when compared to the mutton samples as shown in (Table 3). Most of the *E.coli* strains were resistant to norfloxacin and tetracycline, *Salmonella paratyphi B* strains were resistant to gentamicin, *Salmonella paratyphi A* strains were resistant to Ceftriaxzone and *Salmonella typhimurium* strains were resistant to gentamicin and Co-Trimoxazole (Fig. 1).

Staphylococcus aureus isolates were resistant to tetracycline (Fig. 3) and most of the *Pseudomonas* strains were resistant to gentamicin (Fig. 2)

Table 1: Total percentage of bacterial isolates

Sample	Organism Isolated	Number	Percentage
Chicken	*Escherichia coli*	15	37.5%
	Salmonella paratyphi B	4	10%
	Salmonella paratyphi A	3	7.5%
	Salmonella typhimurium	2	5%
	Shigella flexneri	1	2.5%
	Pseudomonas aeruginosa	4	10%
	Staphylococcus aureus	4	10%
Mutton	*Escherichia coli*	13	32.5%
	Salmonella paratyphi B	4	10%
	Salmonella paratyphi A	3	7.5%
	Salmonella typhimurium	4	10%
	Staphylococcus aureus	6	15%

Table 2: Percentage of total mold isolates

Sample	Organism Isolated	Number	Percentage
Chicken	*Penicillium spp.*	1	2.5%
	Aspergillus niger	1	2.5%
	Mucor spp.	2	5%
	Alternaria spp.	2	5%
Mutton	*Penicillium spp.*	3	7.5%
	Aspergillus niger	1	2.5%
	Aspergillus fumigatus	1	2.5%
	Mucor spp.	1	2.5%
	Sporotrichum spp.	1	2.5%

Table 3: Percentage of total yeast isolates

Sample	*Organism Isolated*	Number	Percentage
Chicken	*Trichosporon spp.*	3	7.5%
	Rhodotorula spp.	2	5%
	Candida spp.	1	2.5%
Mutton	*Trichosporon spp*	2	5%
	Candida spp	2	5%
	Rhodotorula spp.	1	2.5%

DISCUSSION

During a study on the prevalence of *Escherichia coli* in healthy cattle in Switzerland, *Escherichia coli* 0157:H45 strains originating from 6 fattening cattle and 5 cows were isolated. Genotypic, phenotypic and virulence factors (stx, ehxaA, astA, EAF Plasmid, bFp) were described [9]. A minority of *E.coli* strains cause human illness. O157:H7 is a rare *E. coli* serotype that produces one or more toxins that cause damage to the lining of the intestine. In our present study the predominant bacterial pathogen isolated was *Escherichia coli* (70 %). It is quite similar to that of the above study.

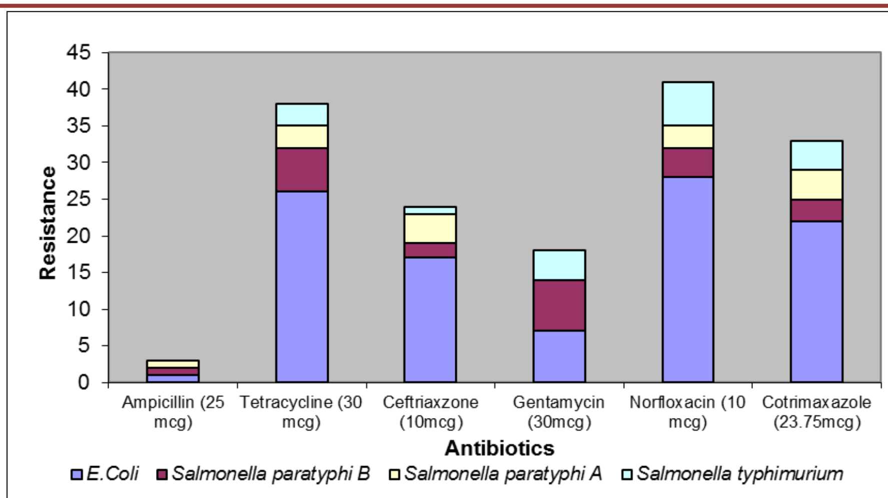

Fig 1: Antibiotic sensitivity pattern for gram negative bacterial isolates

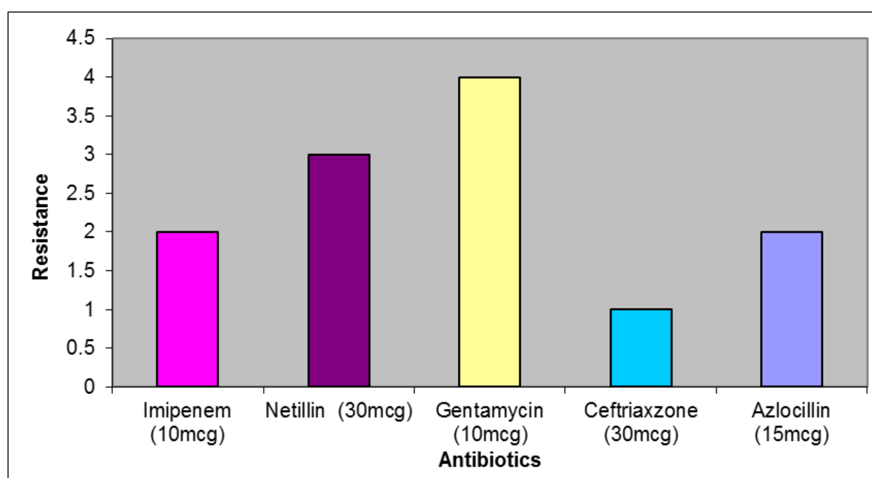

Fig 2: Antibiotic sensitivity pattern for *pseudomonas spps.* Isolates

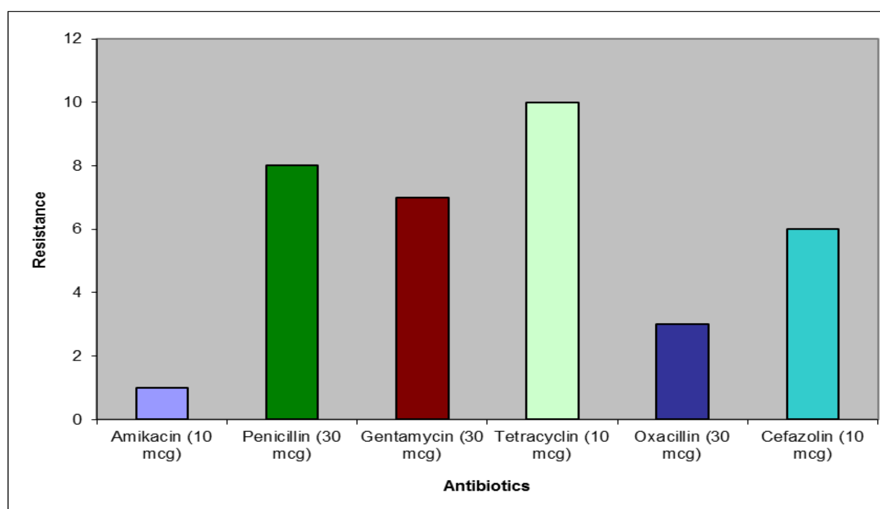

Fig 3: Antibiotic sensitivity pattern for *staphylococcus aureus* isolates

Salmonella spp. remains amongst the most important food-borne pathogens worldwide. In recent years there has been a marked increase in food-borne salmonellosis with outbreaks being reported in several countries including Spain, Italy, England and America. Outbreaks have been linked to a wide range of foods including poultry, eggs, beef, fish, dairy products and chocolate [10]. In our study the percentage of Salmonella isolates were 50%, of which the predominant bacterial pathogen was *Salmonella paratyphi* B (20%), followed by *Salmonella typhimurium* (15%) and *Salmonella paratyphi* A (15%).

In a study MRSA strains were isolated from 264 (11.9%) of 2217 samples analyzed. Isolation percentages for the meat species were: beef (10.6%), veal (15.2%), lamb and mutton (6.2%), pork (10.7%), chicken (16.0%), turkey (35.3%), fowl (3.4%) and game (2.2%). The majority (85%) of the isolated strains belonged to spa-types of pulsed-field gel electrophoresis (PFGE) [11]. Our present study was somewhat similar to that of the above study where *Staphylococcus aureus* was found to be predominant after *E.coli.* The percentage of *Staphylococcus aureus* isolates were 25% of which from chicken sample 10% and from mutton sample 15% was isolated.

Pseudomonas is about 90% of the aerobic spoilage psychrotrophs on meat [12] and had been reported as an inhibitor to some foodborne pathogens**.** However, the *Pseudomonas* used in those investigations were found on fish, pork or chickens and applied to agar assays. Our present study is quite contrasting to that of the above study where the percentage of *Pseudomonas* isolates was only 10%.

Visible moulds were isolated and identified from traditional Greek sausages from Northern Greece. *Penicillium* species were isolated from 90.8% of visibly mouldy sausages. *Penicillium solitum*, *P. nalgiovense* and *P. commune* species made up 60.6% of the total number of isolates [13]. In our present study which is quite similar to that of above studies where the predominant mold pathogen isolated was *Penicillium spp.* (10%), followed by *Mucor spp*. (7.5%), *Aspergillus niger* (5%), *Alternaria spp.* (5%), *Sporotrichum* (2.5%), *Aspergillus fumigatus* (2.5%).

A total of 159 representative yeast isolates obtained from fresh and spoiled processed carcasses were identified according to conventional methods. Species of *Candida, Cryptococcus, Debaryomyces* and *Yarrowia* were isolated from fresh and spoiled carcasses. *Rhodotorula* and *Saccharomyces* spp. were isolated from fresh samples only and *Trichosporon* spp. from spoiled samples only [14]. In our present study the predominant yeast pathogen isolated was *Trichosporon spp.* (12.5%), followed by *Rhodotorula spp.* (10%), *Candida spp*. (5%).

CONCLUSION

The present study reveals the fact that raw meat from retail outlets is heavily contaminated with the high incidence of bacterial pathogens and low incidence of fungal pathogens. The antibiotic resistance pattern of the bacterial isolates shows the high incidence of multi-drug resistant bacterial contaminants in meat. This states the role of raw food as a reservoir of antibiotic resistant bacteria which can be transferred to humans thereby causing gastrointestinal disorders and food borne illness which can be life threatening. It is imperative that basic hygienic practices be incorporated in abattoirs and retail meat outlets to ensure food safety. Training should be given to meat handlers and butchers regarding food safety practices and proper inspection procedures should be strictly adhered to minimise the contamination of raw meat and meat products sold in market places.

Conflict of interest statement

None Declared

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