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PREVALENCE OF ANTIBIOTIC RESISTANCE AND BIOFILM PRODUCING BACTERIAL ISOLATES ON FOODS OF ANIMAL ORIGIN AND FRESH FRUIT JUICES

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ABSTRACT

Antimicrobial resistance is a major global problem for both humans and animals. Increased use of antimicrobials in livestock and poultry was one of the causes of the development of antimicrobial resistance in microorganisms. The aim of the study was to analysis the antimicrobial resistant bacteria from raw meat and fruit juice samples. A total of 7 bacterial genera were recovered from meat and fruit samples. Out of 24 samples, 48 isolates were observed and meat showed the highest number of isolates. *E.coli*, *K.pneumoniae* and *E.faecalis* (21%) were obtained to be the most prevalent bacteria followed by *Proteus* sp (12.5%). Following the prevalence study, antibacterial resistance patterns were observed from all isolates. From the overall results in both sources, among the 11 antibiotics used, penicillin and amoxicillin were resistant to 93.3% of isolates, and a higher number of MDR bacteria were observed in goat samples in comparison to other samples. Additionally, 60.4% of biofilm producers were observed from all samples, especially those which were higher in MDR isolates. This study indicates that antibiotics are at high risk of bacteria, higher in meat than in fruit juices.

Keywords: Meat, fruit juice, MDR, Biofilm, food pathogens

INTRODUCTION

Foodborne diseases are caused by ingestion of contaminated food or drink. Infinite microbes and toxic substances can contaminate foods. There are more than 250 known foodborne diseases all over the world. The majority of infections were caused by bacteria, viruses, and parasites. Other foodborne diseases are essentially poisonings caused by toxins, chemicals contaminating the food. All foodborne microbes and toxins pass through the gastrointestinal tract into the body and often cause the first symptoms, Nausea, vomiting, abdominal cramps and diarrhea are frequent in foodborne diseases.

Foodborne diseases are occurring at any point in the food production, supply and consumption chain. This illness was caused by environmental factors like water, soil, or air pollution, and improper storage and processing of foods. Many microbes can spread in more than one way, so it may not be immediately apparent that a foodborne illness [1].

The foodborne infection was occurred through contaminated raw fruits and vegetables, raw and improperly cooked eggs, poultry and meat, raw milk and milky products that are made with unpasteurized milk. It also can be transmitted through contact with infected animals or infected food handlers who have not hygienic [2].

Food-producing animals (e.g., cattle, chickens, pigs, and turkeys) are the major reservoirs for many foodborne pathogens such as Campylobacter species, non-Typhi serotypes of Salmonella enterica, Shiga toxin-producing strains of *Escherichia coli*, and Listeria monocytogenes [3]. A number of researches have stated the outbreak of infections due to the intake of infected meat and meat products. Karaoke *et al* [4] report that 1.5 billion diarrhea and 3 million deaths worldwide are caused by foodborne infections.

A recent study in India found that bacteria resistant to most potent antibiotics, which were present in fresh food samples including fruits, chicken and fish. This incidence of antimicrobial-resistant is associated with the usage of antimicrobial agents in food producing animals. Food of animal origin carrying a variety of pathogenic and non-pathogenic bacteria has become a vector for interactions which could lead to the creation of new drug-resistant and multidrug-resistant (MDR) bacteria through horizontal exchange of drug-resistant genes, which isolates were not easily eradicated and isolates causing diseases are not easily cured [5 & 6].

Several researchers have reported variable prevalence rates of bacterial infection in different parts of India. The

common antimicrobial-resistant bacteria including *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae*, and *Salmonella* spp., which are the common human normal microbiota as well as important human pathogens, have been reported to be the main forum among foodborne pathogens [7]. In developed and developing countries, the risk of antimicrobial-resistant bacteria affects the economies and health of people.

Both developing countries and developed countries have implemented various national surveillance programs for control of antimicrobial resistance microbes. Nevertheless, some countries completely lack data regarding resistance to antimicrobial agents. Hence the goal of this study was to determine the antibiotic resistance pattern of bacterial isolates from different types of meat and fruit juices collected from in and around the Coimbatore area, TN, India. The findings of this analysis showed that species containing biofilm character and resistant to 6 or more drugs tested.

MATERIALS AND METHODS

Sample collection

A total of 6 food samples each comprising 4 numbers; goat, poultry, and fish meat, in case of fruit juices, apple and orange were collected randomly from four different localities of Coimbatore areas. All samples were aseptically collected and then packaged in sterile polythene zip lock bags and carried to the laboratory in aseptic conditions in a cold box within two hours from the time of purchase.

All samples were analyzed within 2-4 hours after their arrival at the laboratory. A sharp sterile knife was used to cut meat samples from surfaces in sterile trays. To isolate bacteria, homogenate meat samples were streaked onto Chromogenic media, MSA media, SS agar media, blood agar media plates and incubated for 18-24 hours at 35+2 °C. In the case of fruit juices, a loop full of samples was directly inoculated into above mentioned media at incubated for 18-24 hours at 35+2 °C, then genus were confirmed with colony morphology.

Antimicrobial Susceptibility Test

Susceptibility tests were performed using the Kirby-Bauer method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines (NCCLS 2002) [8] and using 11 antibacterial agents.

Determination of biofilm producing isolates

The agar medium was prepared by adding 37g of the BHI powder, 5% of sucrose and 10 g of agar, and Congo red (0.8 g/L) in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121°C. Then the media was poured into the petri plates and allowed to solidify. Once

the media had solidified, the plates were inoculated with the microorganisms and incubated at 37°C for 24 h. The plates were observed the next day, the organisms were considered positive (biofilm-producers) when they produced black colonies on the agar and negative (non-biofilm producers) when they produced pink, or red-orange colonies on the Congo red agar [9].

RESULTS AND DISCUSSION

The distribution of bacterial species per sample is summarized in **Figure 1**. It showed high contamination levels of the sample with total aerobic bacteria and total and fecal coliforms. The biochemical identification confirmed the presence of *E. coli, Salmonella spp, E.faecalis, P.aeruginosa, K.pneumoniae, Proteus spp* and *S. aureus*.

The prevalence of contamination rate was 79.1% in meat samples and 21% in fruit juices samples. Among the meat samples, chicken meat had the highest contamination of 7 bacterial genera (29.1%), and 27% in fish samples. In the case of fruit juices, 12.5% and 8.3% were occurrences in apple and orange respectively. *E.coli, E.faecalis* and *K.pneumoniae* were the dominant isolates and isolated in this study as they were identified in 21% of samples, followed by Proteus spp (12.5%). Shrestha *et al.* [10] made the same finding in 2017; they were

observed the same line of results from chicken meat samples.

During the present analysis, all goat meat samples had different types of bacteria; this level of contamination was significantly lower than in Bhoomika *et al* [11], which reported 46.34% of chevon meat samples containing bacterial isolates. The present study summarized that the distribution of the isolated bacterial strains is different from one butcher shop to another shop; likewise, result was observed in fruit juices. Surprisingly *S.aureus* and Salmonella spp were isolated in limited samples. In 2019 Saud *et al* [6] also found that the minimum percentage of Salmonella spp and *S.aureus* from meat samples. Indeed, the statistical study corroborated with this observation and demonstrates that significant difference (P > 0.05) in contamination rates exists between samples with all the tested bacteria.

While animals are given antibiotics for growth promotion or feed utilization output, bacteria are exposed to low doses of these drugs over a long period of time. This is inappropriate antibiotic use and may lead to resistant bacteria being developed. In this present study, all isolates were subjected to the determination of antibiotic susceptibility with 11 antibiotics.

Number of antibiotic resistance patterns and the subsequently a higher number of MDR bacteria were observed in

goat samples in comparison to other samples. Simultaneously, amongst food isolates, we have no longer found differences in antimicrobial resistance among meats and fruit juices (48.2% and 43% respectively). In meat isolates, Proteus sp was high resistance (58%) and followed by *E.faecalis* (53.3%), in the case of fruit juices isolates, *E.coli* showed high resistance to antibiotics **(Figure 2)**.

A research conducted in samples of chicken meat in Nepal showed a 44% prevalence of Proteus sp, of which 100% isolates were MDR **[6]**. In disagreement with Gaglio *et al*. **[12]**, Sanlibaba *et al*. **[13]**, we detected high resistance to penicillin, amoxicillin and ampicillin. The higher levels of ampicillin resistance in isolates are achieved by the higher levels of penicillin-binding protein 5(PBP5) expressions **[14]**. This antibiotic is frequently used for the treatment of enterococcal infection **[15]**.

Out of 30% *E.coli* isolates from chicken meat samples, 100% were resistant to Amoxicillin, Penicillin, Cefixime and gentamycin, 67% to tetracycline, ampicillin and Novabiocin, 33.3% to Nalidixic acid. Compare to the previous study **[6]**, our result of resistance percentage of Amoxicillin was higher and the same time resistance percentage of Nalidixic acid was low. Out of 30% *K.pneumoniae* obtained from chicken raw meat, 67% were resistant to Nitrofurantoin and Cefixime. The **Figure 3** was revealed the prevalence of antibiotic resistance isolates on food samples. From the overall results, Amoxicillin was resistant to 85% of meat isolates, which was higher than the previous report of Saud *et al* **[6]**. Out of 38 isolates, 39.4% of were MDR isolates, which were highly observed in goat meat samples.
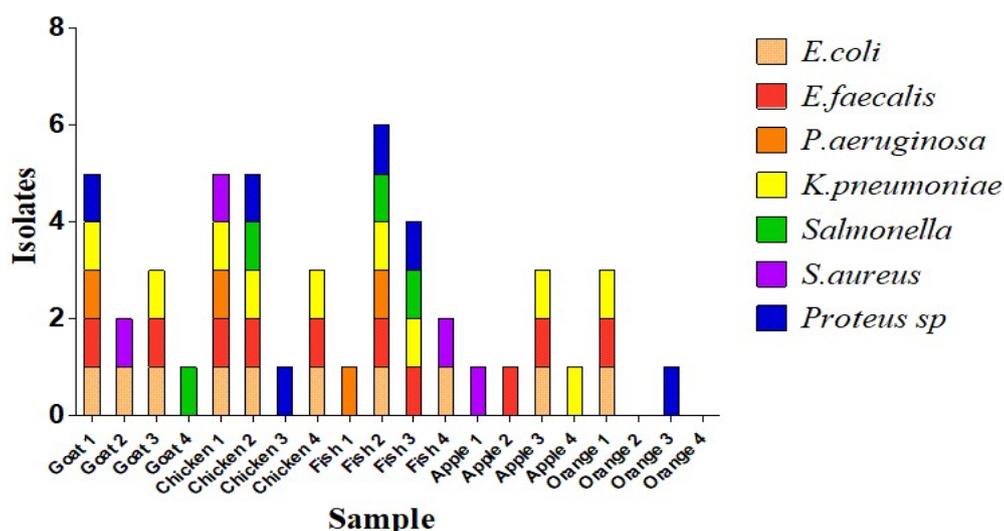

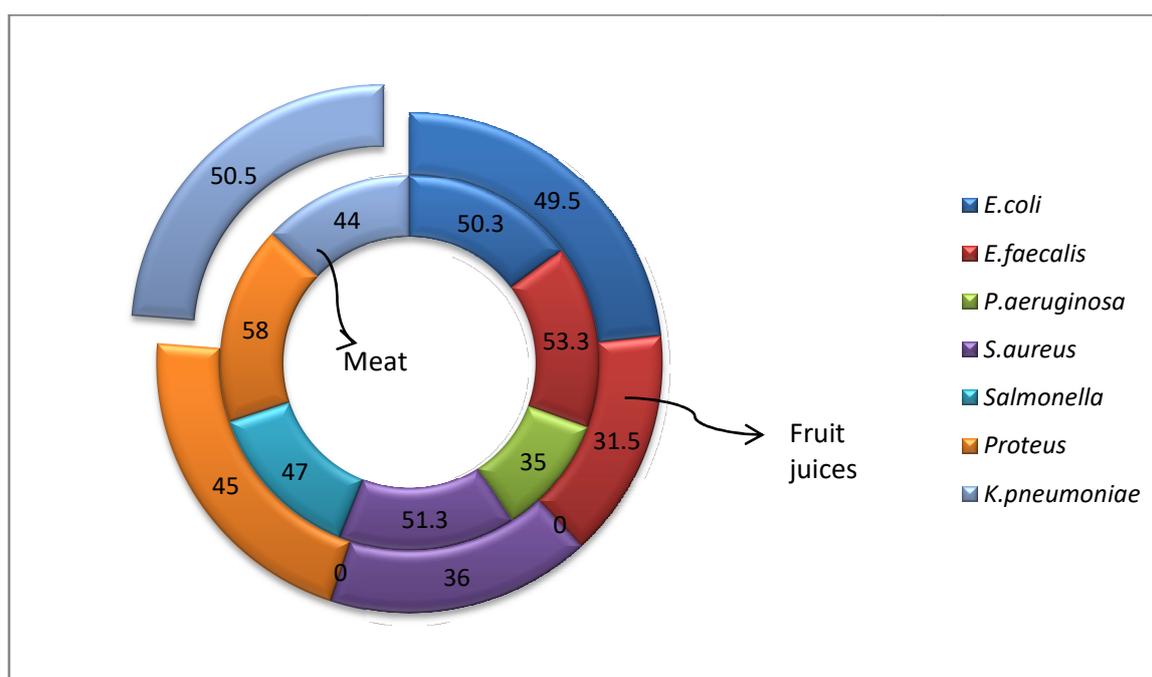**Figure 1: Prevalence of bacterial isolates from food samples**

Figure 2: Prevalence of antibiotic resistance on food isolates
*Outer ring denotes the fruit juice samples and inner ring denote the meat samples

S. No	ISOLATES	TE	K	P	GEN	AMX	C	NA	AMP	NOR	CFM	NV		
	E.coli													100%
	E.faecalis													≤95%
	Salmonella													≤80%
	S.aureus													≤60%
	P.aeruginosa													≤40%
	K.pneumoniae													≤20%
														0%

Figure 3: Percentage of antibiotic resistance on food samples

Following the study of meat, the same effect was observed in fruit juices, and apple and orange juice derived isolates were found to have higher drug resistance, although their size was less than the resistance of the meat-containing isolates. In 2020, Malik *et al*. **[16]** were observed the various bacterial isolates from fruit juices. This is a sign of unhealthy conditions, fruit juices are contaminated with unhealthy methods and poor water usage during or after production.

In this study, the antimicrobial efficacy of the fruit juice was found to be higher for a large number of drugs. Among the 10 of fruit juices isolates, 3 of were multidrug resistance and highest drug resistance against to betalactam antibiotics of penicillin and ampicillin. In 2018, Mandal and Mandal **[17]** was observed the MDR isolates from fruit juices in Malda

Town, India. Other than India, recently in Bangladesh Sultana *et al* [18] was found that Amoxicillin resistance isolates of *K.pneumoniae* from various fruit juices.

From the overall results in both sources, among the 11 antibiotics used, penicillin and amoxicillin were resistant to 93.3% of isolates and Cefixime resistance to 70% of isolates. All isolates except *K.pneumoniae* were 100% resistant to penicillin and amoxicillin. Presently, most of the first line of antibiotics was resistance to food isolates, especially penicillin and amoxicillin. The observed high rates of resistance may be due to uncontrolled consumption, a consequence of easy access to inefficient and cheap antibiotics. The other reason was due to the production of betalactamase enzyme that hydrolyzes the β-lactam ring thereby inactivating the drug [19].

Additionally, biofilm producing isolates were determinate in this present study with congo red agar plate method. Biofilm is bacteria engendering extracellular polysaccharide matrix, which plays a consequential role in evading the host immune system and resisting antimicrobial agents, leading to constant and chronic infections. Among the 48 isolates, 29 (60.4%) of were biofilm producers. The highest biofilm producers were observed from goat (38%) samples and 21% from chicken and fish meat

samples, the lowest percentage (7%) of were from orange juices.

Presently, predominant biofilm producers were *E.coli* and followed by *K.pneumoniae*. Several researches has been recorded the biofilm producing *K.pneumoniae*, and *E.coli* from poultry and goat meat samples [20, 21 & 22]. Comparing with non biofilm producers, biofilm producers possess the highest antimicrobial resistance. This phenomenon was a coincidence with earlier studies [23]. In this study betalactam group of antibiotics (penicillin, ampicillin and amoxicillin) and cephalosporins (Cefixime) were highly resistant in biofilm positive isolates. The study by Marques and colleagues [24] was consistent with our report. His study found that biofilm producing isolates were not controlled by betalactam antibiotics.

In poultry and another animal husbandry, some bacterial species are the key causes of infections. Most of the diseases are related to foodborne outbreaks, live contact with animals, poor hygiene and exposure to the environment. The pathogenesis and virulence of these isolates have increased with the advent of antimicrobial resistance, and treatment options are decreased and are highly costly. Multidrug-resistant bacteria have been detected in meat and fruit samples, which endanger both handlers and consumers and a hazard to public health worldwide. The

above information makes it imperative for the world to monitor antibiotic use in both livestock and humans. The proper antibiotic policy and infection control management in hospital settings is crucial to overcoming the problems associated with infections by MDR strains in humans and animals.

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