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ASSESSMENT OF MULTI-DRUG RESISTANCE AND EXTENDED SPECTRUM BETA LACTAMASE PRODUCING BACTERIA FROM VARIOUS MEAT SAMPLES IN NAMAKKAL, INDIA

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ABSTRACT

The present study was aimed to assess the overall prevalence of MDR and ESBLs from meat samples in Namakkal. A total of 10 (chicken, n=4, goat, n=3 and fish, n= 3) meat samples were studied in which 23 bacteria were isolated. Species of Gram - negative and positive - bacteria were identified as *E.coli* (22%), *P. aeruginosa* (9%), *K. pneumonia* (26%) *Salmonella* spp. (13%), *Proteus* spp. (9%), and *E.faecalis* (22%). The prevalence of MDR isolates was found to be 48%, and a hundred percentage of resistance was against penicillin and lowest resistance to Cefpodoxime. The phenotypic method detected 48% of isolates as presumptive betalactamase producers, however, 39.1% of were as ESBL producers which were by DDST, while using molecular methods detected 55.5%, 78%, 22.2% and 22.2% prevalence of blaSHV, blaTEM, blaCTX-M, and blaOXA respectively. Among the ESBL isolates, 82% were MDR isolates. This showed a wide range of antibiotic - resistant bacteria is prevalent in various meat samples.

Keywords: Biofilm, Beta lactamase, ESBL, bla TEM, *E. coli*

INTRODUCTION

The consumption of contaminated food causes illnesses which are known as food-borne infections or food-borne intoxications in general food-borne diseases sometimes as food poisoning. This acts as a chief health burden throughout the world that ends with high mortality and morbidity the contaminated food causes diarrhoeal disease especially in children by which the infectious diarrhoea leads to 3-5 billion cases accounting for nearly 1.5 million deaths annually. In a study, it was given that the major outbreaks of food-borne infections in India from 1980 to 2016 revealed that the organisms responsible are *Salmonella* sp, *Staphylococcus aureus, Escherichia coli, Yersinia enterolitica, Vibrio* sp and Norwalk-like virus **[1]**.

The vegetables and raw meat are particularly likely to carry the enormous bacteria; one of the possible ways of entry of numerous bacteria could be the handling of contaminated food products by adopting unsuitable hygienic practices during handling and processing **[2]**. According to a recent study in India, it has been identified that bacteria that are resistant to powerful antibiotics were found in fresh food samples that include vegetables, chicken and fish. Antimicrobials are turning into increasingly more ineffective and are posing one of the largest threats to each people and animal **[3]**.

The various antimicrobial resistances, bacterial species like *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae*, and *Salmonella spp* were reported in all over the world and those are the common human normal microbiota as well as important human pathogens, are the major flora among food borne pathogens **[4]**. Infections caused by salmonella sp have been a significant cause of food borne salmonellosis worldwide in recent decades. In India, recently various species of *Salmonella spp* were observed from chicken meat products **[5]**.

Treatment for food borne infection has been increasingly problematic by the emergence of resistance to most first-line of antibiotics **[6]**. Over the years, the resistance of Enterobacteriaceae members to cephalosporins has increased mainly due to the spread of Extended-spectrum β-Lactamases (ESBL). Those isolates have become a serious problem worldwide, which adversely affects the treatment of infectious diseases. Infections with ESBL-producing *E.coli* are associated with a variety of conditions, resulting in increased morbidity, mortality and health care costs **[7]**.

In India, the highest level of cephalosporin resistance *E. coli* has been reported (16–95%) following extensive use of ceftriaxone. In previous review report, 9 of studies were recorded about the ESBL producing poultry meat isolates of *E.coli* and *K.pneumoniae,* which is the result of 2015 to 2019 in India **[8]**. Consumption of these unsafe meats arise the public health hazards. These ESBL producing isolates not only observed from meat samples but also isolated from animal environmental samples and farm workers. Recent studies of Tamta *et al* **[9]** were observed the ESBL producing isolates from fecal and farm workers.

Number studies have investigated the epidemiology of ESBL-producing isolates of animal origins, which may be directly linked to public health. Although, there are inadequate reports on the ESBL producing various meat isolates in the state of Tamilnadu. This study aims to find the prevalence of multidrug resistance, biofilm and ESBL producing isolates from various meats in the Namakkal area. Furthermore, we also verified the existence of any association between drug resistance, biofilm formation and the presence of ESBL.

MATERIALS AND METHODS

Sample collection and isolation of bacteria

A total of 10 samples comprising of chicken meat (4), goat meat (3) and fish meat (3) were collected from plastic covers and transported quickly to the laboratory. One gram of samples was transferred to conical flasks containing peptone water and incubated for 30 min at 80 rpm at room temperature in a rotator. After incubation, samples from peptone water were streaked into chromogenic and SS agar media. All the plates were incubated aerobically at 37 °C for 24 h. All isolates were identified by following standard microbiological techniques which include studies of colony morphology and staining reactions. Pure isolates were identified by performing the standard biochemical tests (IMVIC and sugar fermentation test) **[10]**.

Antibiotic susceptibility test

Antibiotic susceptibility test of isolates was performed following a modified Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) **[11]**.

Screening of betalactamase producing isolates by tube method

Penicillin solution was dispensed in 0.5ml volume in small test tubes. Test bacteria were removed with a loop from an overnight culture on solid medium and suspended in the Penicillin solution (1000U) to give a density of at least

10^4CFU/ml. After one hour at room temperature, two drops of starch indicator were added to the suspension, followed by one drop of Iodine reagent. The positive reaction was indicated by the disappearance of the blue color immediately. Persistence of blue color for longer than 10 minutes constituted a negative test **[12]**.

Phenotypic Confirmatory Test for ESBL Production

Mueller-Hinton agar culture medium was used to perform this study. Four different antibiotic discs were used, which included cefotaxime (30 µg), cefotaxime/clavulanic acid (30 µg/10 µg), and Amoxicillin disc (30µg) alone and in combination with clavulanic acid (30µg/10 µg). The study was performed in accordance with the study of Ghazaei, **[13]** and Harwalkar *et al*., **[14]**.

Isolation of Biofilm producing isolates

The agar medium was prepared by adding 37 g of the BHI powder, 50 g of sucrose and 10 g of agar in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121°C. Once the agar solution was cooled down to about 50°C, a solution of Congo red (8 g/L) was added and mixed again. Then the media were 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 **[15]**.

PCR amplification for the detection of beta lactamase genes

All isolates were screened for the resistance genes SHV, TEM, CTX-M, and OXA by a multiplex PCR assay using Hong *et al*., **[16]** procedure. The amplified PCR products were subjected to electrophoresis at a 1.5% agarose gel in 1XTBE buffer. The 1000bp molecular weight marker was used to measure the molecular weights of amplified products.

RESULTS AND DISCUSSION

Out of 7 samples received during the study period, only 23 were gram-negative and positive isolates were distributed as *E. coli* (22%), *K.pneumoniae* (26%), *P.aeruginosa* (9%), *E.faecalis* (22%), *Salmonella sp* (13%) and *Proteus sp* (9%). Among the 23 isolates, 69.5% of were gram-negative and 30.4% of were gram-positive. Presently, the highest bacterial occurrence was observed in chicken samples. A similar observation was found by Shrestha *et al*., **[10]**. They were also observed the various species from chicken meat samples.

During the present study, all samples of goat meat had various bacteria genera, this level of contamination was significantly higher than compared to reports of Bhoomika *et al* **[17]**, who recorded 46.34% of Chevon meat samples harbor the bacterial isolates. In 2018, Nagarajan *et al*., **[18]** were observed the various bacterial genera from poultry meat, goat meat, and fish meat. These bacterial contamination mainly occurred by the unhygienic condition of butchers shop. The meat was cut on filthy wooden logs (tree trunks) that are never washed and dried in the sunlight. The butchers are not used to wearing gloves and most diseases today are food borne due to bacterial contamination. Most of the people buy meat from local slaughter shops which pose a high risk of contamination.

In a study of antimicrobial susceptibility, it must be pointed out that some significant differences regarding antimicrobial resistances in certain antibiotics were observed, depending on the origin of the isolates. Simultaneously, among food isolates, we have not found huge differences in antimicrobial resistance between chicken, goat and fish meats (43.5%, 45% and 44.4% respectively). Presently all isolates were resistant to penicillin and 88% of isolates were resistant to erythromycin. Recently from Northeast Algeria, penicillin resistances of various bacterial isolates were observed from meat samples **[19]**. It is not surprising that there is no perfect antibiotic, and antibiotic use always involves compromise. Additionally penicillin was not suggested for human because it causes side effect and the common occurrence of resistance **[20] (Table 1)**.

Presently, cefotaxime was resistant to 68% of isolates, among them 78% of isolates from poultry meat and 75% of from goat meat samples. Among the 6 genera, 3 of were showed 100% resistance to cefotaxime (*P.aeruginosa, Salmonella sp* and *Proteus sp*). Waghamare *et al*., **[21]** were observed the 14.19% of cefotaxime resistance in *Salmonella sp*, which were observed from poultry meat and poultry related products.

In this study, lowest resistance was against to cefpodoxime (12%) ceftriaxone (12.5%) antibiotic and followed by gentamycin (13%). This occurrence was lower than previous Moawad *et al*., **[22]**, who were observed the 20% of Cefpodoxime and Ceftriaxone resistance isolates from poultry meat samples. Out of the 6 types of bacterial genera, there is no large difference in the resistance ratio among the 4 types of genera, namely, *E.coli* (47%), *E.faecalis* (47%), *Salmonella sp* (47.3%) and *Proteus sp* (46%). Based on

the result of bacterial genera, 0% of Cefpodoxime resistance was observed in *E.coli, K.pneumoniae*, *Salmonella sp* and *Proteus sp*. This was not coinciding with the results obtained by Moawad *et al*., **[22]**, they were observed that the highest range of Cefpodoxime resistance *E.coli* from poultry meat samples.

The study of antimicrobial susceptibility points out a high resistance against penicilinese group of penicillin and second most was Macrolide group of erythromycin. The 34.5% of isolates were resistant to the Aminoglycoside group of antibiotics. In the case of isolates wise, *E.coli* had 100% resistance against amikacin. Similar results were detected in a study carried out in Spain **[23]**. Moreover, presently 13 types of resistance patterns were observed from 23 isolates, among them 7 of were grouped and 6 of were none grouped **(Figure 1)**.

Biofilm are bacteria producing extracellular polysaccharide matrix, which plays an important role in avoiding the host immune system and resisting antimicrobial agents, leading to persistent and chronic infections. Our results indicated that most of the examined isolates were positive for biofilm. In our tested strains, 61% were identified to be biofilm producers. Among them, the highest prevalent isolates were *E.coli* (17.3%) and following bacterial species were shared for 2nd place, such as *K.pneumoniae, E. faecalis* and *Salmonella spp.*

Several studies were observed the biofilm - producing *K.pneumoniae, Salmonella sp* and *E.coli* from chicken and goat meat samples **[24-26]**. In case of source wise, the highest prevalence of biofilm producers was in chicken samples. Comparing with non biofilm producers, biofilm producers possess highest antimicrobial resistance. This phenomenon was a coincidence with earlier studies **[27]**. We found an exception that the resistance rate of penicillin was higher in non-biofilm producing isolates also. Our Study on the biofilm-forming traits of meat isolates could help us recognize the increasing resistance to antibiotics in meat isolates as well as their pathogenicity to host.

Food animals are increasingly being identified as a reservoir for ESBL producing strains. Studies from around the world have shown that ESBL generating isolates such as *E.coli* and *Klebsiella* can contaminate foods of animal origin and make contributions to illnesses and spoilage. The present study revealed that beta lactamase producing isolates were observed in the meat samples. Totally 39.1% of isolates were observed by iodometric method. **Figure 2** illustrate that the prevalence of biofilm and

betalactamase producing isolates from meat samples.

Furthermore, those positive isolates were subjected to a double - disc diffusion method for evaluating of ESBL positive isolates. The results of the initial screening of iodometric test were in accordance with the results obtained from the complementary confirmatory test of ESBL, with 90% of the isolates being positive in both analytical tests.

Presently, predominant beta lactamase producers were observed in chicken samples, in the case of species wise, *E.coli* (60%) showed highest producers. A number of studies have reported an increased ESBL case from *E. coli* strains isolated from animals **[8, 10]**. According to the study from 2013 to 2019 have been increased ESBL isolates, in our analysis of this process are reflected, for instance, in previous studies, the species-wise prevalence of ESBLs was found to be 9, 10 and 5% for *E.coli*, *K. pneumoniae,* and *Pseudomonas* spp. respectively **[8]**. At the same time, in the present study, 60%, 17% and 33.3% of were observed respectively. The MDR pattern of *E.faecalis* and Proteus sp also were ESBL producers. Numerous studies have reported the presence of MDR strains *E.faecalis* and Proteus species from animal sources **[28, 29]**.

Table 1: Percentages of resistance against different antibiotics according to isolates origin

Antibiotics	Origin of strains		
	Goat meat	Chicken meat	Fish meat
P	100	100	100
CTR	37.5	0	0
CPD	25	22.2	0
G	0	22.2	17
CAZ	37.5	55.5	67
AK	62.5	55.5	50
TE	12.5	11.1	33.3
CFM	62.5	55.5	50
E	75	89	100
COT	0	11.1	33.3
CTX	62.5	7	50
NIT	50	33.3	33.3

P-Penicillin, CTR- Ceftriaxone, CPD- cefpodoxime, G-Gentamycin, CAZ- ceftazidime, Ak-Amikacin, TE-Tetracycline, CFM- cefixime, E- Erythromycin, COT- Co-trimoxazole, CTX- Cefotaxime, NIT- nitrofurantoin

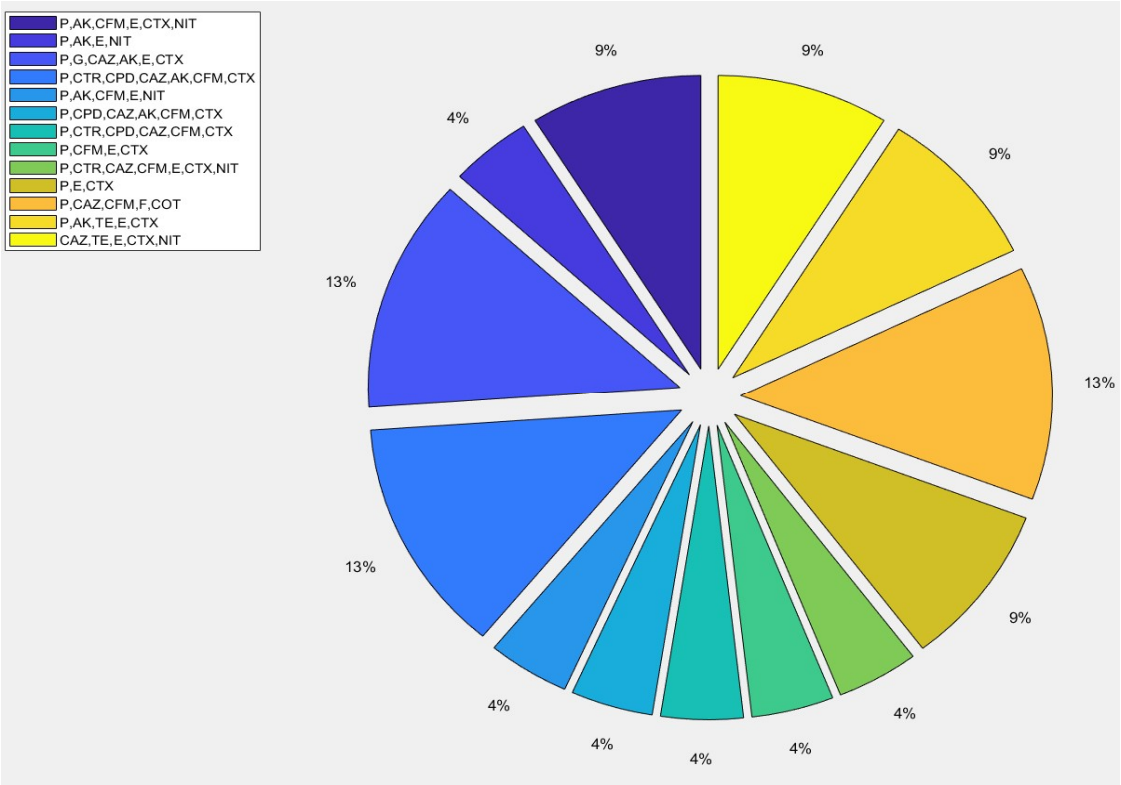

Figure 1: Antimicrobial Resistance Patterns on meat Isolates

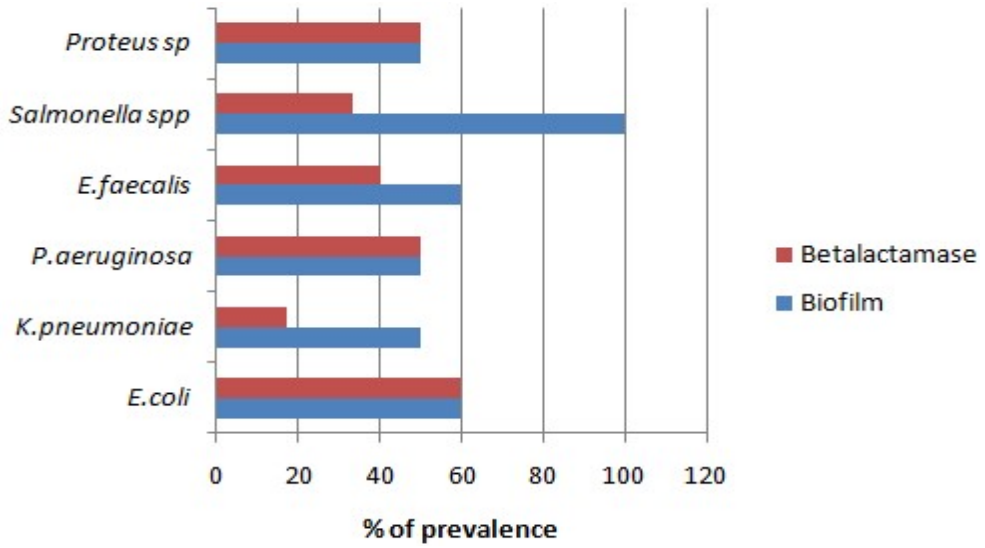

Figure 2: Prevalence of biofilm and beta lactamase producing meat isolates

At present, animals without any recognized risk factor for MDR isolates are found to have ESBL-producing organisms. Therefore, an evaluation of ESBL-producing isolates has become important. Despite the number of methods, the effective results are found by the molecular characteristics which methods are reliable and accurate then phenotypic method. In the present study, phenotypically positive

isolates were subjected to PCR for amplification of ESBL genes. The overall prevalence of blaSHV, blaTEM, blaCTX-M and blaOXA genes among isolates recorded was 55.5%, 78%, 22.2%, and 22.2% prevalence respectively. The prevalence rate of the blaTEM gene was highly observed in chicken meat sample isolates. Similarly, Apaka *et al*. **[30]** and Bhoomika *et al*., **[17]** were also reported a higher prevalence of blaTEM gene from chicken samples. Occasionally, multiple genes in single isolates are responsible for the development of ESBL. Therefore, presently multiplex PCR was used for the detection of ESBL isolates, because while using this M-PCR, simultaneously the number of genes was amplified.

Overall, we found a prevalence (45%) of multidrug resistance and ESBLs producing isolates in all meat samples. It is well documented that both Gram-negative and positive isolates harbor series of antibiotic-resistant genes which can be transferred to other bacteria horizontally. This indicates the advent of MDR meat strains is a powerful threat. This phenomenon mainly occurred by due to low sanitary widespread of butcher stores and unhygienic practices of meat handlers. Hence, improve the knowledge and practice of butchers about handling and processing of meat. Additionally, adequate antibiotic policies and infection control schemes in hospital settings are vital to overcome the problems associated with ESBL-producing isolates in humans.

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