



Microbial diversity of carbapenemase producing Enterobacteriaceae from poultry farm and slaughter house sample around Namakkal area

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Abstract: The poultry industry is one of the industries that is expanding at a rapid rate all over the world. Food borne infections, also known as food poisoning, are any illnesses that are brought on by the consumption of contaminated food or beverages. Food poisoning is another common name for food borne infections. One of the leading causes of death across the globe is this infection, which is a public health problem. The contamination of raw meat is one of the primary causes of illnesses that are transmitted through food. Through the handling of raw poultry carcasses and products or through the consumption of poultry meat that has not been cooked to the appropriate temperature, enterobacteriaceae can be transmitted to humans. Poultry and poultry products are constantly contaminated with enterobacteriaceae, which can be transmitted to humans. Bacteria, and more specifically *Camphylobacter*, *Salmonella*, and *E. coli*, are the primary agents that are responsible for the majority of cases of bacterial gastroenteritis that affect humans all over the world.

Index Terms: Poultry farm, slaughter house, SS agar Karmili agar and MBL genes.

I.INTRODUCTION

In India, there is a growing demand for poultry and products derived from poultry. In comparison to the prices of other meat products available on the market, the prices of poultry products are offered at competitive prices. The primary economic drivers for the grill industry have been factors such as the production of live stock, efficiency, the health of flocks, and the quality of dead animals and meat. The poultry industry in India has been expanding at a rate of approximately 8-10% annually over the past decade, with grill meat volumes growing at a rate of more than 10%. On the other hand, because of increased domestic consumption, table egg production has been at a rate of 5-6%. (Sivanantham *et al.*,2023)

The meat of chicken is more popular among consumers due to its easy digestibility and acceptance by the majority of people. However, it is possible for chicken meat to be contaminated with a wide range of potentially pathogenic food borne pathogens that can cause human diseases. These pathogens include *Salmonella* spp,

Campylobacter jejuni, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* spp, *Pseudomonas* spp, and *Listeria* spp (Khalafalla *et al.*, 2015). According to Lister and Becker (2010), there are approximately 325,000 cases that require hospitalization and approximately 5000 cases that result in fatalities each year. Food poisoning affects approximately twenty percent of the population in England, according to the findings of the most extensive study ever conducted on infectious intestinal disease, which was conducted in the United Kingdom. (Abubakar *et al.*, 2007).

According to Chuma *et al.*, (1997), the presence of pathogenic and spoilage microorganisms in poultry meat and its by-products continues to be a significant concern for suppliers, consumers, and public health officials all over the world. Poultry and poultry products are constantly contaminated with enterobacteriaceae, which can be transmitted to humans through the handling of raw poultry carcasses and products or through the consumption of poultry meat that has not been cooked to the appropriate temperature. On account of the fact that salmonella is typically found in poultry, this particular type of meat has been an important vehicle in the transmission of food-borne diseases. Salmonellosis is one of the food-borne diseases that is reported the most frequently all over the world. (Khalafalla *et al.*, 2015).

According to the findings of research conducted over the course of the past three decades, *Campylobacter* control on poultry farms continues to be elusive. As a result of the majority of broiler flocks becoming infected by the third or fourth week of rearing, the approaches that are currently being utilised are ineffective (Patriarchi *et al.*, 2009; Thangaraj and Sivanantham, 2015). These approaches are heavily dependent on biosecurity. According to Sudershan *et al.*, (2010), Indhu *et al.*, (2014), Indhu *et al.*, (2017), and Indhu *et al.*, (2023), food borne illnesses are caused by improper agricultural practices, poor sanitary and hygiene conditions at all stages of the food, a lack of preventive controls in food processing operations, improper mixing of food additives and chemicals, and improper storage and handling of the food. In recent decades, there has been a rise in the prevalence of antimicrobial resistance among pathogens that are transmitted through food. (Angulo *et al.*, 2000).

II. MATERIALS AND METHODS

Collection of samples

Two different poultry farms and slaughterhouses for poultry were used to collect samples from various locations within Namakkal. Within the test tube that contained sterilized peptone water, four distinct samples were collected at random from each poultry farm (cage, faeces, meat, and water) and slaughter house (meat, cage dehairing machine (blender), and chopping board). These samples were collected using cotton swabs that had been sterilized. As a result, a total of twenty samples were analyzed, which were collected from poultry farms and slaughterhouses.

Preparation of samples

The samples were cleaned with sterile cotton swap from the cage, the chopping board, and the dehairing machine. After that, they were inoculated into peptone broth and then incubated at 37° for 24 hours. Once the samples were inoculated into the nutrient broth, one gm of meat samples were homogenized for three minutes with normal saline. Through the use of the colony color and morphology on the chromogenic agar, SS agar, and Karmili agar, a presumptive identification of the isolates was accomplished. *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Proteus species*, and *E. feacalis* were all isolated using this chromogenic medium.

The isolation of the bacterium *Campylobacter jejuni*

Every sample was placed on a Karmali *Campylobacter* Agar plate that had been sterilized, and then it was incubated with 10% carbon dioxide at 37° for 24 hours. Following the incubation period, the colonies of a greyish color were observed on the plate. In order to isolate salmonella species, each and every broth sample was inoculated into a medium consisting of sterile SS agar solution. Following an incubation period of 24 hours, colonies of a dark color were observed, which were identified as being *salmonella* species.

Isolation of MBL producing isolates through the use of the phenotypic method

The bacteria isolates that were being tested were swabbed in an aseptic manner onto Muller-Hinton (MH) agar plates. Additionally, standard antibiotics discs consisting of imipenem (10 µg) and impregnated with EDTA (1 µg) were placed on the MH agar plates in an aseptic manner. It was ensured that there was a distance of 25 mm between each antibiotic disc. An inference of MBL production can be made phenotypically if there is a difference of at least seven mm between the zones of inhibition of any of the carbapenems with EDTA and discs that do not contain EDTA.

Clinical isolates for the purpose of plasmid separation

The alkaline lysis procedure was utilized, with some modifications here and there, in order to successfully isolate plasmid DNA. It was decided to transfer one ml of a culture that had been growing overnight into an eppendorf tube. This was accomplished by centrifuging the cells for a short period of time at a speed of 5000 revolutions per minute in the microfuge, and then draining the supernatant. For the purpose of resuspending the pellet, 100 µl of Solution A was added, and the contents were thoroughly mixed using a vortex. After that, 100 µl of solution B was added, and the contents were thoroughly mixed by inverting them four to five times. All of the DNA pellets were dissolved using 20 µl of TE buffer after the supernatant was drained off. Using agarose gel electrophoresis, the DNA of the plasmid that was extracted was validated.

Agarose gel electrophoresis

The electrophoresis of agarose gel was performed in a horizontal submarine electrophoresis unit throughout the experiment. After preparing sixty ml of 1% Agarose gel with 1X TBE buffer, the contents were heated to the point where they became a clear solution for the purpose of casting Agarose gel. After the solution had been allowed to cool, a staining dye solution containing 5 ml was introduced into the casting system. After the addition of 10 ml of plasmid DNA, it was combined with 2 ml of gel loading dye and then loaded onto the gel. Following the connection of the power card terminals at their respective positions, the gel was run at a voltage of fifty volts until the gel loading dye had migrated more than half the length of the gel. Following that, the unit was turned off, and the DNA that had been isolated was observed using a UV transilluminator.

Amplification of the gene for metallo beta-lactamase

Taq DNA polymerase, deoxynucleoside triphosphate, and gene specific primers were utilized in order to carry out multiplex PCR amplification for the purpose of simultaneously amplification of the blaIMP and blaVIM metallo beta-lactamase genes. The reaction mixture consisted of the following components in its composition: Each PCR reaction mixture totaling 25 µl contained 2 µl of template DNA, which was plasmid DNA, 10 µl of 10 X PCR mix, 0.5 µl of each primer with a concentration of 0.5 mM, and 12 µl of molecular grade water. The PCR programme was carried out in a Thermal Cycler, and it included an initial incubation that lasted for ten minutes at 37°, followed by an initial denaturation step that lasted for five minutes at 94°. After that, there were thirty cycles of DNA denaturation at 94° for one minute, primer annealing at 54° for one minute, and primer extension at 72° for one to three minutes.

III.RESULT

The purpose of sampling, two distinct slaughterhouses and poultry farms were selected from the vicinity of Namakkal. In each of the selected slaughterhouses and poultry farms, two samples were collected in a separate manner (Tables 1 and 2). A total of 18 samples were collected, seeded on the peptone broth, and then incubated at 37° for 24 hours. The poultry farms had received 8 samples, and the abattoir had received 5 samples.

Isolates of bacteria that are prevalent

A colour reaction was observed on the Chromogenic media for the bacterial isolates that were obtained from the poultry farm and the abattoir. Every single one of the 18 samples that were examined exhibited significant growth, which included growth that was both unimicrobial and polymicrobial in nature. The colour of the colony was used as a basis for the detection of pathogens on chromogenic media. A selective medium consisting of Karmali agar was utilised in this investigation for the purpose of isolating *Camphylobacter* species. *Camphylobacter jejuni* is the organism that is indicated by the grey ash colour. The presence of *Salmonella* species was indicated by the presence of the black colour that was observed in *Salmonella shigella* agar. (Table 3).

After conducting this research, it was discovered that both of the samples contained eight different types of bacterial species. In both samples, the highest percentage of bacterial isolates was found to be *Enterococcus faecalis* and *Camphylobacter* spp, which accounted for 19.5% of the total. *Pseudomonas* spp, which accounted for 15.2% of the total, came in second place, followed by *Salmonella* spp, *Proteus* spp, and *K. pneumonia*, which accounted for 6.5% of the total. The *E. coli* percentage was the lowest, coming in at 4.3%.

Finding betalactamase producing isolates

In this study, betalactamase test was executed with tube test. Among the 26 isolates, 12(46.1%) of were positive, which were decrease the blue colour while including of iodine solution. The prevailing isolates were observed in chopping board and followed cage and blender samples. The betalactamase creators weren't detected from meat sample isolates. In case of poultry farm isolates, 11 (55%) of were positive for betalactamase producers, which were strongly predominate in stool tests separates (Table 4 and Table 5).

Diagnostic MBL phenotype

The purpose of this investigation was to identify metallo betalactamase (MBL) by selecting imipenem resistant isolates. There was zone formation as a result of the EDTA disc synergy test because EDTA inhibits MBL from the imipenem resistant strain. There were a total of 55% and 62 point 5 % of isolates that were produced from poultry farm and abattoir production, respectively. (Table 6).

Gene amplification for MBL producing

In this particular investigation, the imipenem resistance of seventeen different isolates was selected for PCR analysis in order to further amplify the MBL genes (IMP and VIM). In total, 88.2 percent of the isolates tested positive for the presence of MBL producers. Sixty-four percent of the seventeen isolates contained a single gene, while four of them harboured two genes, namely SMP, FCE, SBEF, and SCEF. The results of this study showed that two of the isolates did not produce any types of genes. The majority of MBL genes were found in the isolates obtained from slaughterhouses, followed by those obtained from poultry sample isolates. These two types of isolates were evaluated. (Table 7 and Plate).

IV.DISCUSSION

The microorganisms that are transmitted to humans through food products are among the most important vehicles for this transmission. Among these microorganisms, enterobacteriace is still a major cause of food borne human disease in the majority of regions around the world. *Salmonellae* are frequently found in poultry and poultry products, and they can be transmitted to humans through the handling of raw poultry carcasses and products, as well as through the consumption of poultry meat that has not been cooked thoroughly. (Bonyadian *et al.*, 2007 and Sasikala *et al.*, 2018).

Bacteria, particularly *Camphylobacter*, *Salmonella*, and *E. coli*, are the primary agents responsible for the majority of cases of bacterial gastroenteritis that affect humans around the world. The duration of the symptoms, which typically last for up to a week, includes fever, abdominal cramps, and diarrhea. However, the infections frequently resolve on their own and do not necessarily call for the use of antibiotic drug treatment. Antibiotic

treatments that are effective are essential and beneficial for the progression of the disease, but they are only considered necessary in severe cases. In addition, conditions such as Guillain-Barre syndrome and reactive arthritis have been linked to infections caused by *Campylobacter*. (Altekruse *et al.*, 1999).

A primary cultural examination was performed on all 18 samples that were seeded on chromogenic media and then incubated at a temperature of 42° for a period of 48 hours in the current study. The isolates demonstrated the growth of organisms that had colonies of coloured colonies that were distinctive. Out of the 18 samples, seven different bacterial genera were found to be present in both types of samples. Of these seven genera, five were found to be present in chromogenic media, and the remaining two genera were *salmonella* and *Camphylobacter* spp., which were isolated using SS agar and *Camphylobacter Karmali* agar, respectively. There were a total of 46 isolates that were found, with *E. feacalis* and *Camphylobacter* spp. having the highest distribution, and *Pseudomonas* spp. having the second highest distribution.

Salmonella has been found in chopping boards and knives, according to Mukul *et al.*, (2012) investigation. On the other hand, a study conducted in retail meat shops discovered a higher level of *Salmonella* contamination with chopping boards 36.0% of the time. The chopping boards were also found to be highly contaminated, followed by knives, which can be compared to the findings that we have presented here (Thiruppathi *et al.*, 2004). The fact that different kinds of meat were kept and sold from the same counter almost certainly contributed to an increase in the amount of contamination that occurred. It is highly likely that cross contamination will take place if meat that has been stored comes into contact with other meat that is contaminated or with equipment that is contaminated.

One of the most common reasons for bacterial resistance to β -lactam antibiotics is the production of enzymes known as β lactamases. In recent years, there has been a notable rise in the level of bacterial resistance to β -lactam antibiotics for antibiotics. The spread of plasmid-mediated extended spectrum β -lactamases (ESBLs) has been characterized by this increase, which has happened in a consistent manner. In the majority of cases, ESBLs are found in the family of Enterobacteriaceae. *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas serotypes*, and *Salmonella serotypes* are the primary species in which ESBL enzymes have been reported the most frequently across the world. These enzymes are responsible for between five and twenty percent of the outbreaks of nosocomial infections that occur in intensive care units.

The effective detection and recognition of MBLs is essential for the implementation of effective countermeasures to limit the spread of organisms that carry these enzymes and for the appropriate treatment of infections that are caused by microorganisms that produce MBLs. There are a few phenotypic methods that have been published for the detection of MBL; however, the results have shown that no method alone is able to detect all of these enzymes. This is most likely due to the genetic variability of these enzymes.

V. CONCLUSION

The conclusion that can be drawn from the current study with poultry-related products is that the percentage of bacterial isolates was higher than expected. In addition, the majority of the isolates exhibited resistance to antibiotics that were classified as fluoroquinolones and quinolones. When it comes to the treatment of human gastroenteritis infections, these antibiotics are generally regarded as the primary medications of choice. In light of these findings, it appears that the consumption of meat that has not been properly cooked or food that has been cross contaminated with bacteria, particularly *K. pneumoniae*, *Salmonella* spp, and *Campylobacter* spp, may constitute a significant risk to the health of consumers. Due to the fact that the majority of the isolates in this study exhibited high levels of resistance, it was difficult to eradicate these isolates. As a result, it is of the utmost

importance that we find a new way to reduce the problem and develop research for new drugs that are derived from natural products.

Table 1

Occurrence of bacterial species on poultry slaughter house materials

| S.No | Name of the samples | No.of samples | No. of bacterial spp | Name of the Bacterial speices |
|------|---------------------|---------------|----------------------|--|
| 1. | Meat | 2 | 5 | <i>Enterococcus faecalis, Proteus spp, Pseudomonas aeruginosa, , shigella spp, Camphylobacter jejuni.</i> |
| 2. | Fecus | 2 | 5 | <i>Enterococcus faecalis, Pseudomonas aeruginosa, Shigella spp, Salmonella spp, Camphylobacter jejuni.</i> |
| 3. | Cage | 2 | 5 | <i>Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella spp, Camphylobacter jejuni.</i> |
| 4. | Chopping board | 2 | 7 | <i>E.coli, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella spp, Salmonella spp, Camphylobacter jejuni.</i> |
| 5. | Blender | 2 | 4 | <i>Enterococcus faecalis, pseudomonas aeruginosa, Shigella spp, Camphylobacter jejuni.</i> |

Table 2: Occurrence of bacterial species on poultry farm materials

| S.No | Name of the samples | No.of samples | No. of bacterial spp | Name of the Bacterial speices |
|------|---------------------|---------------|----------------------|--|
| 1. | Meat | 2 | 5 | <i>E.coli, Enterococcus faecalis, Proteus spp, Shigella spp, Camphylobacter jejuni.</i> |
| 2. | Feces | 2 | 6 | <i>Enterococcus faecalis, , proteus spp, Pseudomonas aeruginosa, Klebsiella pneumonia Shigella spp, Camphylobacter jejuni.</i> |
| 3. | Cage | 2 | 5 | <i>Enterococcus faecalis, Pseudomonas aeruginosa, Shigella spp, Salmonella spp</i> |

| | | | | |
|----|-------|---|---|---|
| | | | | <i>Camphylobacter jejuni.</i> |
| 4. | water | 2 | 4 | <i>Enterococcus faecalis,</i> <i>Pseudomonas aeruginosa,</i> <i>Shigella spp, Camphylobacter</i> <i>jejuni</i> |

Table 3
Morphological characterization of bacterial isolates on various media

| S.No | Isolates | Morphology/Colour |
|------|---|-------------------------|
| 1. | <i>E.coli</i> | Pink colony |
| 2. | <i>Klebsiella pneumonia</i> | blue colony with mucoid |
| 3. | <i>Pseudomonas aeruginosa</i> | Colour less colony |
| 4. | <i>Proteus vulgaris</i> | Brown colony |
| 5. | <i>Enterococcus faecalis</i> | Small blue colony |
| 6. | SS agar- <i>Salmonella spp</i> | Black colour |
| 7. | SS agar <i>Shigella spp</i> | Pink colour |
| 8. | Karmali agar <i>Camphylobacter spp</i> | Ash colour colony |

Table 4
Isolation of betalactamase producing slaughter house isolates

| S.No | Isolates name | Betalactamase result |
|------|---------------|----------------------|
| 1. | SMEF | - |
| 2. | SMP | - |
| 3. | SMPA | - |
| 4. | SMSH | - |
| 5. | SMC | - |
| 6. | SFEF | + |
| 7. | SFPA | - |
| 8. | SFSH | - |
| 9. | SFS | + |
| 10. | SFC | - |
| 11. | SCEF | + |
| 12. | SCPA | - |
| 13. | SCK | - |
| 14. | SCSH | + |
| 15. | SCC | + |
| 16. | SCBE | - |
| 17. | SCBEF | - |
| 18. | SCBPA | + |
| 19. | SCBK | - |
| 20. | SCBSH | + |
| 21. | SCBS | + |
| 22. | SCBC | + |
| 23. | SBEF | - |
| 24. | SBPA | + |
| 25. | SBSH | + |
| 26. | SBC | + |

Table 5
Isolation of betalactamase producing poultry farm isolates

| S.No | Isolates name | Betalactamase result |
|-------------|----------------------|-----------------------------|
| 1. | FME | + |
| 2. | FMEF | - |
| 3. | FMP | - |
| 4. | FMSH | + |
| 5. | FMC | - |
| 6. | FFEF | + |
| 7. | FFP | + |
| 8. | FFPA | + |
| 9. | FFK | + |
| 10. | FFSH | - |
| 11. | FFC | + |
| 12. | FCEF | + |
| 13. | FCPA | + |
| 14. | FCSH | - |
| 15. | FCS | + |
| 16. | FCC | + |
| 17. | FWEF | - |
| 18. | FWPA | - |
| 19. | FWSH | - |
| 20. | FWC | - |

Table 6

Isolation of MBL producing isolates by phenotypic method

| Sample | Isolates name | Results for MBL |
|-----------------|---------------|-----------------|
| Poultry farm | FME | + |
| | FMSH | + |
| | FFEf | - |
| | FFP | - |
| | FFC | + |
| | FCEf | - |
| | FCPA | + |
| | FCC | + |
| Slaughter house | SFS | + |
| | SCEf | + |
| | SCC | + |
| | SCBSH | + |
| | SCBS | - |
| | SCBC | - |
| | SBPA | + |
| | SBSH | + |
| SBC | - | |

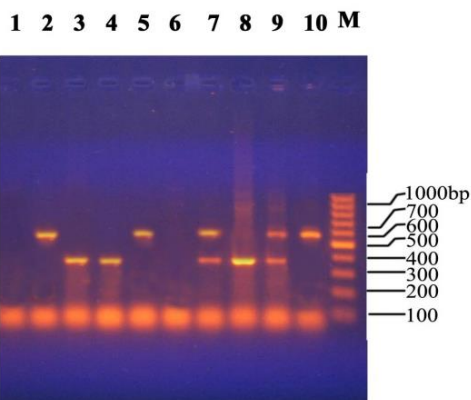


Table 7

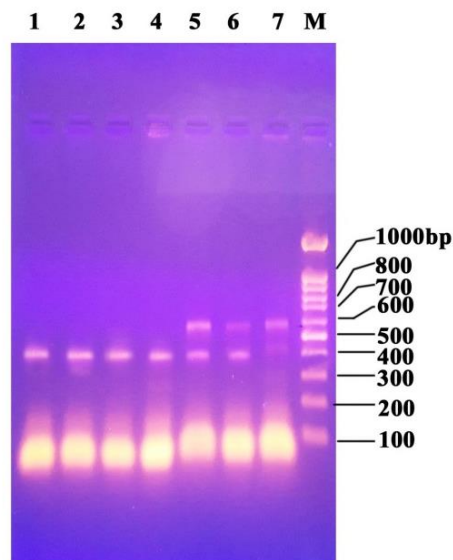
Amplification of MBL genes by multiplex PCR

| S.No | Isolates name | MBL genes | |
|------|---------------|-----------|-----|
| | | VIM | IMP |
| 1. | FCS | - | - |
| 2. | SCBE | + | - |
| 3. | SCBK | - | + |
| 4. | FFP | - | + |
| 5. | SMEF | + | - |
| 6. | SCBEF | - | - |
| 7. | SMP | + | + |
| 8. | FCPA | - | + |
| 9. | FCE | + | + |
| 10. | SCBSH | + | - |
| 11. | FME | - | + |
| 12. | FCC | - | + |
| 13. | FFK | - | + |
| 14. | FFSH | - | + |
| 15. | SBEF | + | + |
| 16. | SCEF | + | + |
| 17. | SFEF | + | - |

Amplification of MBL genes from slaughter house and poultry farm isolates



**1-FCS, 2-SCBE, 3-SCBK, 4-FFP, 5-SMEF, 6-SCBEF, 7-SMB, 8-FCPA, 9-FCE
10-SCBSH, M-100bp DNA marker**



**1-FME, 2-FCC, 3-FFK, 4-FFSH, 5-SBEF, 6-SCEF
7-SFEF, M- 100bp DNA marker**

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