RESEARCH ARTICLE

Study on Prevalence and Antibiotic Profiling of *Enterococcus* Spp. from Chicken Meat

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Abstract

The present study was undertaken to characterize *Enterococcus* species isolated from poultry meat by cultural isolation, PCR detection and antibiogram. Antibiotic profiling of detected *Enterococcus* spp. isolates were done by disc diffusion method. Total 150 chicken meat samples were examined for the presence of *Enterococcus* spp. The overall prevalence of *Enterococcus* spp. by cultural isolation was 56%. The prevalence of *Enterococcus* spp. by targeting tuf gene and ddlE.faecalis gene in PCR was 95.23 and 77.38%, respectively. In the antibiotic sensitivity test, *Enterococcus* isolates showed high resistance toward Erythromycin and Tetracycline. However, low resistance was seen towards Chloramphenicol, whereas all the isolates were susceptible to Gentamicin. This study found a high prevalence of multi-drug resistance among enterococci isolated from poultry meat, suggesting that these resistant bacteria and genes may be transported from food to humans and pose a significant risk to human health in the coming years.

Keywords: Antibiotic resistance, Chicken meat, *Enterococcus*, PCR.

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Introduction

Enterococci are ubiquitous bacteria normally occurring in foods, particularly those of animal origin, such as meat and milk and fecal contaminant indicator (Cattoir, 2022). The Genus *Enterococcus* consists of Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic bacteria that can occur both as single cocci and in chains and belong to the Lancefield group D *streptococci* (Fisher and Phillips, 2009). *Enterococci* are traditional commensals within the gut of poultry and lower hygienic standards in poultry slaughtering compared with another animal slaughtering can result in more contamination of poultry meat. Poultry meat could also become contaminated with *Enterococcus* species during food handling.

Presently, they are among the top three pathogens causing nosocomial infections, leading to morbidity and mortality (Moreno *et al.,* 2006). *Enterococcus* species, particularly *E. faecium* and *E. faecalis*, are important in public health. In humans, they have been implicated as an important cause of endocarditis, bacteraemia, infections of the urinary tract, central nervous system, intra-abdominal and pelvic infections, and multiple antibiotic resistances. Its treatment has become difficult due to the increasing number of antibiotic-resistant E*nterococci* (Poh *et al.,* 2006). The utilization of antimicrobial agents in poultry production has been widely documented (Butaye *et al.,* 2003). So, it has been recommended that enterococci isolated from foods ought to be tested in terms of potential antibiotic resistance. Hence, this study aimed to characterize *Enterococcus* species from poultry meat by cultural isolation, PCR and its antibiogram.

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MATERIALS AND METHODS

Sample Collection

150 poultry meat samples were collected under aseptic precautions from the various retail meat shops in Anand city of Gujarat, India. The standard strain of *Enterococcus faecalis* Microbial Type Culture Collection (MTCC 439) used as reference in the study was obtained from Chandigarh.

Isolation and Identification of *Enterococcus* Species

Enrichment of all the samples was carried out in Buffered Peptone Water (BPW) enrichment broth. Ten gram sample

was homogenized with 90 mL sterile BPW and then incubated at 37± 1°C for 24 h. The selective media used to isolate Enterococcus spp. was Slanetz-Bartly (SB) Medium and Citrate Azide Tween Carbonate (CATC) agar. A loopful of inoculum was taken from enrichment broth and was streaked on SB medium and CATC medium. The inoculated plates were then incubated for 24-48 h at 37°C for SB medium and 18-24 h for CATC medium. The appearance of characteristic red or maroon colored small colonies on SB agar and red colonies on CATC media were considered as presumptive Enterococcus spp. The pure cultures were then streaked on Brain Heart Infusion agar (BHI) and incubated for 24 h at 37°C and these colonies were utilized for all further procedures (Pangtey, 2017).

Confirmation and Antibiotic Profiling of the *Enterococcus* spp.

The Enterococcus isolates were subjected to *in vitro* antibiotic sensitivity test by standard disc diffusion method on Muller-Hinton agar according to Bauer *et al.* (1966). Isolates were tested for antimicrobial sensitivity against 8 antibiotics and were classified as sensitive, intermediate, and resistant according to guidelines provided by Clinical Laboratory Standard Institute (CLSI, 2017). Antibiotics used in this study were Ampicillin (AMP,10 µg) , Chloramphenicol (C,30 µg**)**, Ciprofloxacin, (CIP, 05 µg), Gentamicin (GEN, 10 µg) , Vancomycin (VAN, 30 µg) , Erythromycin (ERY,15 µg), Rifampicin (RIF, 05 µg) , Tetracyclin (TE, 30 µg) .

Culturally positive isolates of Enterococcus spp. were subjected to molecular characterization using PCR for confirmation by targeting genus and species-specific tuf gene and *ddlE.faecalis* gene, respectively.

PCR Protocol

The DNA from *Enterococcus* isolates were extracted by snapchill method of DNA extraction. The amplification protocols of PCR for tuf gene and ddlE.faecalis gene were standardized as described by Kasimoglu-Dogru *et al.* (2010) and Dutka-Malen *et al.* (1995), respectively. The oligonucleotide primers used for the tuf gene of genus Enterococcus and ddlE.faecalis gene of *E. faecalis* is given in Table 1.

The reaction mixture for PCR was prepared in 200 µL PCR tubes. The reaction mixture contained 12.5 µL PCR mastermix (2X), 1-µL each of forward and reverse primer (10 pmol), 5.5 µL nuclease-free water and 5 µL templates. For tuf gene cycling conditions for PCR included an initial denaturation of DNA at 94°C for 5 min, followed by 30 cycles of 94°C for 30s denaturation, 50°C for 90s annealing, 60s extension at 72 °C, followed by the final extension of 10 min at 72°C and hold at 4°C. The cycling conditions for ddlE.faecalis gene included an initial denaturation of DNA at 94°C for 10 min, followed by 30 cycles of 94°C for 15s denaturation, 55°C for 15s annealing, 45s extension at 72°C, followed by the final extension of 05 min at 72°C and hold at 4°C. The final amplified product was analyzed by agarose gel electrophoresis on 2% agarose gel and visualized under gel documentation system.

RESULTS AND DISCUSSION

Isolation of *Enterococcus* from Chicken Meat

The primary cultural method used for isolation (Fig. 1 and 2) of bacteria from 150 poultry meat samples revealed the overall prevalence of *Enterococcus* spp. as 56% (84/150). Similar finding of around 62.5% prevalence was reported by Banik *et al.* (2018). In contrast, a high prevalence rate of 91.60% was observed by Naas *et al.* (2021) may be due to unhygienic conditions followed during the processing of meat (Laban *et al.*, 2021). However, low prevalence rate of 39.21% (40/102) was reported by Talebi *et al.* (2015), which may be due to good hygienic practices.

Out of 84 positive isolates of Enterococcus, 80 (95.23%) isolates were found to be positive for tuf gene (112 bp) by PCR (Fig. 3). The present finding is almost similar to the report of Manson *et al.* (2019). They revealed 98.23% prevalence rate of Enterococcus spp from chicken meat, while a relatively low prevalence rate of 79.50% was also reported by Sanlibaba *et al.* (2018). On the other hand, Rozanska *et al.* (2015) reported moderate prevalence rate (49%) of Enterococcus spp. However, a very low prevalence rate of 30% was reported by Onaran *et al.* (2019), which may be due to good hygienic practices followed in sampling.

Amongst the *Enterococcus* isolates from poultry meat, 77.38% (65/84) were detected for *ddlE.faecalis* gene (941

Table 1: Description of primer used for detection of Enterococcus spp.

Target gene	*Primer sequence (5' — 3')*	*Product Size (bp)*	*Reference*
Tuf	Ent1-TACTGACAAACCATTCATGATG Ent2-AACTTCGTCACCAACGCGAAC	112	Ke *et al.* (1999)
ddlE.faecalis	F: ATCAAGTACAGTTAGTCTT R: ACGATTAAAGCTAACTG	941	Dutka-Malen *et al.* (1995)

Fig 1: Red or maroon colonies of Enterococcus on Slanetz-Bartly (SB) Medium

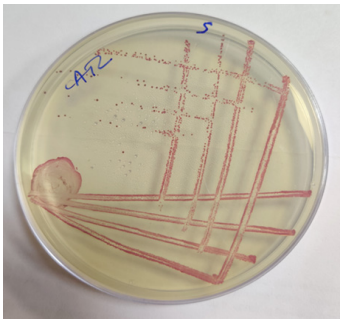

Fig 2: Red colonies of Enterococcus on Citrate Azide Tween Carbonate medium (SB) Medium

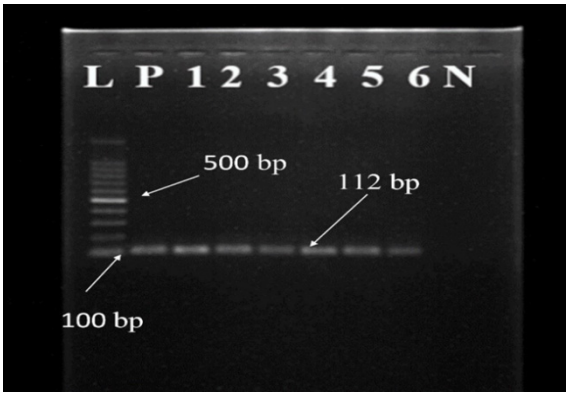

Fig 3: Agarose gel showing amlification product of tuf gene (112 bp)

bp) by specific PCR (Fig. 4). In contrast to our finding the high prevalence rate (94.50%) was reported by Kim *et al.* (2018) and a moderate prevalence rate (44.4%) was reported by Onarana *et al.* (2019). However, low prevalence rate of only 9.3% was reported by Gousia *et al.* (2015). These differences in prevalence could be attributed to geographical discrepancies or to different isolation methodologies. The presence of enterococci in meat products can be considered an indicator of fecal contamination during evisceration in slaughterhouses (Zelalem *et al.*, 2022).

Antibiotic Resistance Profiling of *Enterococcus* spp.

Enterococcus isolates showed high degree of resistance towards the antibiotics Erythromycin (85.71%) and Tetracycline (77.38%), while moderate resistance was observed towards Ampicillin (64.28%), Ciprofloxacin (47.61%) and Rifampicin (46.42%). Furthermore, an intermediate resistance was observed towards Vancomycin, and a low resistance toward Chloramphenicol (25%), whereas all the isolates were susceptible to Gentamicin (Fig 5). This may be due to differences in the ecological features for microbial growth and antibiotics usage in veterinary practices of the specific region (Rozanska *et al.*, 2015).

CONCLUSION

The current study revealed the presence of enterococci in chicken meat in Anand, Gujarat. In addition, a high level

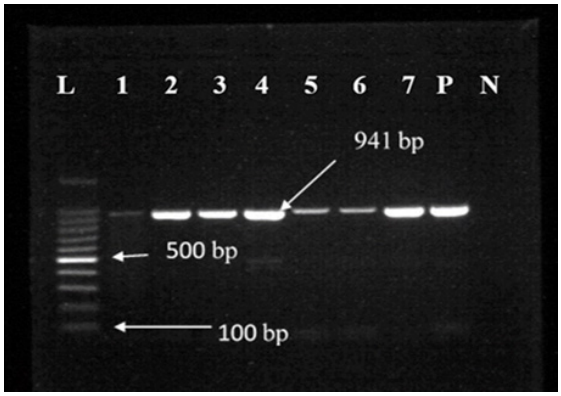

Fig 4: Agarose gel showing amplification product of ddlE.faecalis gene (941 bp)

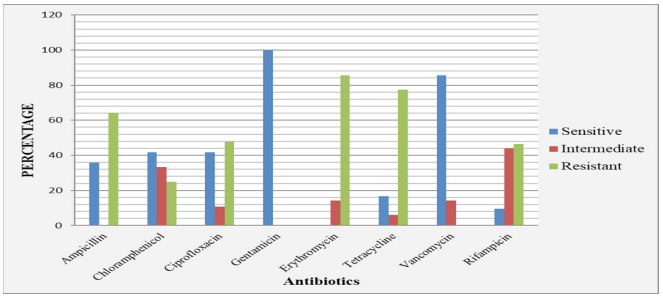

Fig.5: Antibiotics sensitivity/resistance pattern of Enterococcus spp.

of resistance to a clinically important class of antibiotics was discovered in Enterococci, raising concerns about the effective treatment of infections as well as the potential transfer of this resistance to other intestinal organisms. Further research is needed to establish the risk of transmission of these organisms from foods of animal origin to humans and to monitor the increasing antimicrobial resistance and resistance genes.

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