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Prevalence of virulence associated genes in *Enterococcus* spp. isolates from chicken meat

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Abstract
Present study was conducted to detect the prevalence of major virulence genes of *Enterococcus* spp. from raw chicken meat. Total 80 isolates of the *Enterococcus* spp. were recovered from the 150 chicken meat sample by Polymerase Chain Reaction (PCR) and all the isolates were further screened for virulence associated genes, namely aggregation substance (*asa*), enterococcal surface protein (*esp*), cytolysin (*cyl*), gelatinase (*gelE*), hyaluronidase (*hyl*). Study revealed that prevalence of *gelE* gene was comparatively high (88.75%) followed by *asa gene* (40.47%), *esp* gene (15%) and *cylA* gene (11.25%). None of the *Enterococcus* spp. isolates harbor *hyl* gene.

Keywords: *Enterococcus*, PCR, virulence genes, chicken meat

1. Introduction
Enterococci are Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic bacteria, which usually inhabit the alimentary tract of humans in addition to being isolated from environmental and animal sources (Fisher and Phillips, 2009) [2]. Many virulence factors have been discovered in enterococci, some of which are considered very important in the pathogenesis of diseases caused by them. While clinical *Enterococcus* strains have been extensively described in the literature, the knowledge of the virulence factors and the genetic structure of enterococci found in food are limited. Knowledge of the virulence characteristics of *Enterococcus* strains may help to understand the complex pathogenic process of these opportunistic microorganisms (Sharifi *et al*., 2012) [10].
Enterococci are capable of adhering to their host's tissues (Tomita and Ike, 2004), resistance to low pH scale and high concentrations of bile salts (Moreno *et al*., 2006) contribute to enterococci being the most prominent among all bacterium colonizing the colon. Their adhesins alter them to bind to receptors on the mucous membrane or to proteins of the extracellular matrix that favour colonization of the epithelium (Franz *et al*., 2003). Colonization itself is not proof of pathogenicity, however, combined with other factors of virulence and with the presence of a number of resistance genes, potentially harmful.
Virulence factors that promote colonization include aggregation substance, collagen-binding protein, cell wall adhesin, and enterococcal surface protein. After the colonization process, pathogenic strains of *Enterococcus* species secrete toxic substances that have a damaging impact on the host's tissues. Virulence factors secreted by enterococci include cytolysin, gelatinase and hyaluronidase (Hollenbeck and Rice, 2012) [3].

2. Materials and Methods
2.1 Sample Collection
A total of 150 poultry meat samples were collected under aseptic precautions from the various retail meat shops in sterile sample collection vials from Anand, Gujarat, India

2.2 Isolation and Identification
Enrichment of all the samples was carried out in Buffered Peptone Water (BPW) enrichment broth. The selective media used for isolation of *Enterococcus* spp. was Slanetz-Bartly (SB) Medium and Citrate Azide Tween Carbonate (CATC) agar. The appearance of characteristic red or maroon coloured 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 hours at 37 ºC and these colonies were utilized

for all the further procedures. The isolates suspected to be *Enterococcus* spp. was subjected to morphological and biochemical tests. Culturally and biochemically positive isolates of *Enterococcus* spp. were subjected for molecular characterization and virulence genes detection by PCR.

2.3 Detection of Virulence Associated Genes of *Enterococcus* spp.

The isolates of *Enterococcus* spp. were screened for the detection of major virulence-associated genes by targeting *asa*1, *gelE*, *cyl*A, *esp* and *hyl* genes (Table 1). The DNA from isolates was extracted by boiling method. A loopful of pure culture was suspended in 100 µl of DNAse and RNAse free Milli-Q water in a sterilized eppendorf tube. The suspension was vortexed and then heated at 95 ºC for 10 min in the thermal cycler. This was then centrifuged at 10000 rpm for 6 min in order to settle down the cell debris. The upper aqueous phase was transferred to another eppendorf tube and this was used as a DNA template for PCR**.** The reaction mixture was prepared in 200 µl PCR tubes which contained 12.5µl PCR master mix (2X), 1 µl each of forward and reverse primer (10pmol), 5.5 µl nuclease free water and 5 µl templates. The details of thermal profiling of PCR are mentioned in Table 2 described by Pangtey (2017) [8] with slightly modifications. The final amplified product was analysed by agarose gel electrophoresis on 2% agarose gel and visualized under gel documentation system.

Table 1: Description of primer used for detection of virulence associated genes of *Enterococcus* spp. (Vankerckhoven *et al*., 2004)

Sr. No.	Target-Gene	Primer sequence (5' — 3')	Product Size (Base pairs)
1.	*Asa*	F: GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375
2.	*gelE*	F: TATGACAATGCTTTTTGGGAT R: AGATGCACCCGAAATAATATA	213
3.	*cylA*	F: ACTCGGGGATTGATAGGC R: GCTGCTAAAGCTGCGCTT	688
4.	*Esp*	F: AGATTTCATCTTTGATTCTTGG R: AATTGATTCTTTAGCATCTGG	510
5.	*Hyl*	F: CAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	276

Table 2: PCR condition for detection of virulence associated genes

Cycling Conditions		Temperature	Time
Initial Denaturation		94 ºC	2 min
35 cycles	Denaturation	94 ºC	1 min
	Annealing	55.5 ºC	2 min
	Extension	72 ºC	1 min
Final Extension		72 ºC	10 min

3. Results and Discussion

All the 80 positive isolates were subjected to PCR for confirmation (Parmar *et al*., 2022) [9] and virulence associated genes of *Enterococcus* species confirmed by PCR. For these, virulence genes namely *asa*, *gelE*, *cyl*, *esp* and *hyl* was targeted with PCR procedure as described by Pangtey, 2017 [8]. The prevalence of *asa* gene in the present study was found to be 40.47% (34/80) which was lower than the prevalence (90.47% and 63.10%) reported by Hosseini *et al.* (2016) [4] and by Kim *et al.* (2018) [5], respectively (Fig 1).

The prevalence of *gelE* gene in the present study was 88.75% (71/80) which was quite similar to the findings recorded by Yılmaz *et al*. (2016) [13] and Kim *et al*. (2018) [5] who reported 82.90% and 95.30% prevalence rate, respectively (Fig 2). Comparatively lower prevalence (31.19%) was recorded by Molechan *et al*. (2019) [6].

The prevalence of *esp* gene in the present study was 15.0% (12/80) which was lower than the prevalence (50.0% and 54.60%) reported by Hosseini *et al*. (2016) [4] and Youssef *et al*.(2019) (Fig 3). The prevalence of *cyl* gene in the present study was 11.25% (9/80) (Fig 4) which was higher than the prevalence (2.70% and 3.0%) reported by Kim *et al*. (2018) [5] and Molechan *et al*. (2019) [6], respectively. High prevalence (33.90%) was reported by Youssef *et al*. (2019). In the present study the prevalence of *hyl* gene was 0.0% which was almost similar to the findings of Kim *et al*. (2018) [5] who reported a very low prevalence of 01.34%.

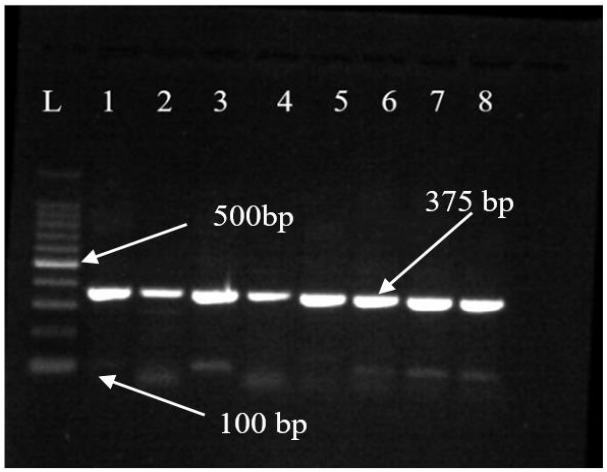

Fig 1: Agarose gel showing amplification product of *asa* gene (375 bp)

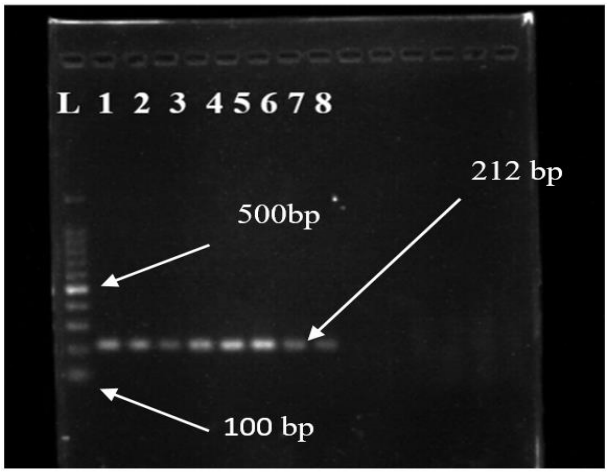

Fig 2: Agarose gel showing amplification product of *gelE* gene (212 bp)

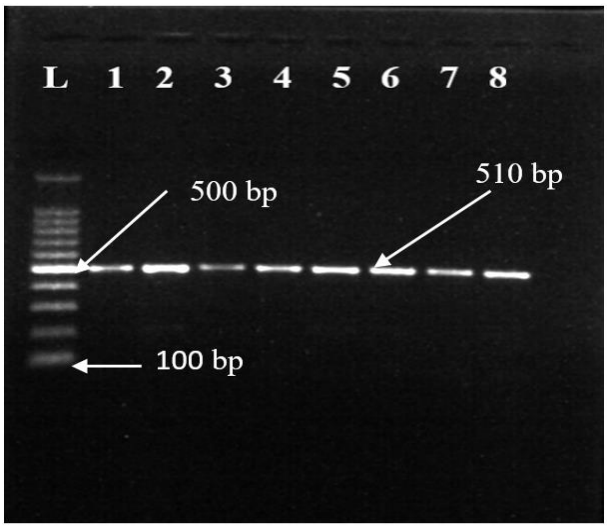

Fig 3; Agarose gel showing amplification product of *esp* gene (510 bp)

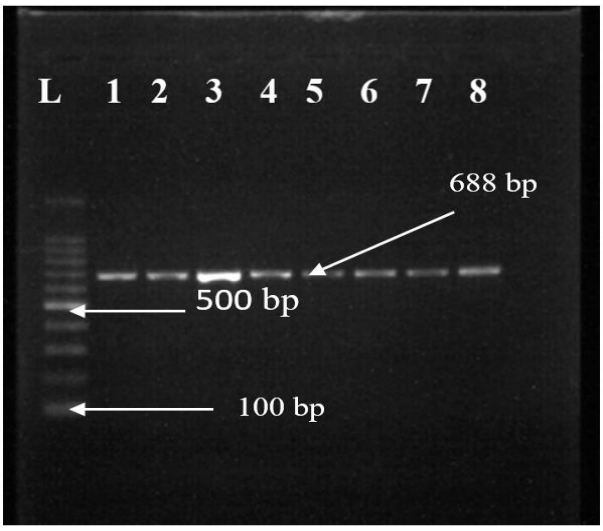

Fig 4: Agarose gel showing amplification product of *cyl gene* (688 bp)

4. Conclusion

In conclusion, the current study reveals the presence of virulence genes of Enterococci in raw chicken meat Anand, Gujarat. The presence of virulence genes does not necessarily mean that the strains recovered from the foods cause illness in humans, but may have pathogenic potential as these factors have been found to contribute to the severity of infection. Although factors of virulence are less frequent in isolates from food than in isolates of clinical origin, they can be a potential reservoir of virulence factors and they can make it possible to transfer them to human microbiota in the food chain. Therefore, significance of enterococci in foods still represent a challenge and should come under tighter control.

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6. References

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