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Research article

Assessment of prevalence, antibiotic resistance, and virulence profiles of biofilm-forming *Enterococcus faecalis* isolated from raw seafood in Bangladesh

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ABSTRACT

Enterococcus faecalis are often resistant to different classes of antibiotics, harbor virulence determinants, and produce biofilm. The presence of *E. faecalis* in raw seafood exhibits serious public health significance. This study aimed to identify antibiotic resistance patterns and virulence factors in biofilm-forming *E. faecalis* strains extracted from seafood in Bangladesh. A total of 150 samples of raw seafood, comprising 50 shrimps, 25 crabs, and 75 fish, were collected and subjected to culturing, biochemical, and PCR assays to detect *E. faecalis*. The biofilm-forming abilities of the isolates were determined by Congo Red agar (CRA) plate and Crystal Violet Micro-titer Plate (CVMP) tests. Antibiotic resistance profiles were evaluated using the disk diffusion method. Virulence genes of the isolates were detected by PCR assay. The occurrence of *E. faecalis* was 29.3 % (44/150), which was higher in crabs and fish (36 %) than in shrimps (16 %). In CRA and CVMP tests, biofilm-forming abilities were observed in 88.64 % of the isolates, whereas 11 (25 %) and 28 (63.6 %) were strong- and intermediate-biofilm formers, respectively. All the isolates contained at least two virulence genes, including *pil* and *ace* (97.7 %), *sprE* (95.5 %), *gelE* (90.9 %), *fsrB* (79.6 %), *agg* (70.5 %), *fsrA* (68.2 %), and *fsrC* (61.4 %). All the isolates were phenotypically resistant to penicillin, followed by ampicillin and rifampicin (86.4 %), erythromycin (13.7 %), and tetracycline, vancomycin, norfloxacin, and linezolid (2.3 %). Resistant gene bla_{TEM} was found in 61.4 % of the isolates. Moreover, the study found that *E. faecalis* strains with strong biofilm-forming capabilities had significantly higher levels of virulence genes and antibiotic resistance ($p < 0.05$) compared to those with intermediate and/or no biofilm-forming abilities. To the best of our knowledge, this research represents the first instance in Bangladesh of assessing antibiotic resistance and identifying virulence genes in biofilm-forming *E. faecalis* strains isolated from seafood samples. Our study revealed that seafood is a carrier of antibiotic-resistant, virulent, and biofilm-forming *E. faecalis*, demonstrating a potential public health threat.

1. Introduction

Enterococci are among the frequently occurring bacterial groups in various types of food, including seafood. These bacteria are

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widely spread and frequently inhabit the gastrointestinal tracts of humans and farm animals, with *E. faecalis* being particularly prevalent [1–3]. Their robust survival capabilities in diverse conditions, including high temperatures, salinity, and acidity, contribute to their widespread presence in different environments [4]. They can easily acclimate to the gastrointestinal environments of their hosts and are commonly found in soil, water, and surrounding areas [5]. Moreover, they can be present in various raw materials derived from both plants and animals, such as meat, vegetables, and milk [6]. The occurrence of *Enterococcus* spp. in foods, including seafood, is mostly attributed to these bacteria's ability to adapt to harsh environmental circumstances in relation to production and storage procedures.

Enterococcus faecalis stands out as a major culprit in infections acquired within hospital settings, posing significant risks, particularly to individuals with compromised immune systems [7]. This bacterium is capable of instigating severe infections, spanning from meningitis and bacteremia to urinary tract and gastrointestinal infections, as well as periodontitis [8]. The urinary tract, bloodstream (particularly in newborns), cardiac, surgical wound, intra-abdominal, pelvic, and, occasionally, the nerves are the most often infected locations by this organism [9].

Bacteria frequently utilize biofilms as a survival mechanism against harsh environmental conditions, disinfectants, and antimicrobial substances [10,11]. Enterococci, in particular, are renowned for their capacity to form biofilms, wherein clusters of cells firmly attach to various surfaces, including both biotic and abiotic materials [12,13]. Biofilms play a significant role in developing enterococcal infections [14]. Moreover, specific toxins and virulence factors in these bacteria raise the intensity and harmfulness of diseases that they can transmit [15]. Several genes, including gelatinase (*gelE*), serine protease (*sprE*), quorum-sensing (*fsrA*, *fsrB*, and *fsrC*), pili (*pil*), cytolysin (*cyl*), gene connected to enterococcal adhesion to collagen (*ace*), aggregation substance (*agg*), and others, are significant virulence factors responsible for various aspects of these bacteria's behavior, including adhesion, colonization, evasion, enzyme extracellular synthesis, biofilm growth, pathogenicity, and severity of recurrent infections [15,16]. In addition, these bacteria are capable of competing with other microorganisms, colonizing the host, evading the body's immune defenses, and causing pathological alterations, either by producing toxins or tolerating aggravation [17].

Antimicrobial resistance (AMR) is a major and escalating public health concern, hindering both human and veterinary medicine by reducing antibiotic effectiveness and complicating bacterial infection treatment [18,19]. There has been a recorded increase in the number of antibiotic-resistant *Enterococcus* strains throughout time [20]. Enterococcal strain safety assessments are challenging due to the high occurrence of resistance and virulence factors [21]. Managing enterococcal infections can pose challenges because these organisms naturally resist various antimicrobial classes like cephalosporins, aminoglycosides, macrolides, and sulfonamides [22]. Hence, adopting a holistic "One Health" perspective is imperative to recognize the diverse elements, encompassing human, animal, and environmental factors, contributing to the increasing levels of AMR [23].

The existence of commensal microorganisms in environmental ecosystems [24] and in food and human environments [25] suggests that the food chain may be vital in the transmission of AMR and virulence factors between the environment and humans [26]. Seafood samples may harbor strains of *E. faecalis* with heightened levels of virulence and resistance [27]. Seafood as a balanced, healthful diet has recently gained more interest in many areas in Bangladesh. While previous studies in Bangladesh have explored enterococci in different sources [10,12,28–38], there remains a dearth of information concerning AMR and virulence traits in biofilm-forming *E. faecalis* specifically isolated from seafood. In our previous study [10], we showed the presence of biofilm-forming *E. faecium* in seafood samples. This study aimed to address these gaps, recognizing the lack of available data in Bangladesh regarding AMR and virulence characteristics of biofilm-forming *E. faecalis* in seafood.

2. Materials and methods

2.1. Ethical considerations

The ethics committee at Bangladesh Agricultural University, Mymensingh, Bangladesh, approved all the protocols and methodologies related to the present study (AWEEC/BAU/2023(25)).

2.2. Sample collection and processing

One-hundred and fifty raw seafood samples (50 shrimp, 25 crabs, and 75 sea fish) were collected from different retail markets of the Moheshkhali (21.5374° N, 91.9418° E), Cox's Bazar Sadar (21.4272° N, 92.0061° E), and Kutubdia (21.8167° N, 91.8583° E) upazilas areas within the Chittagong region of Bangladesh. Twenty-five white shrimp (*Penaeus indicus*) and 25 tiger shrimp (*Penaeus monodon*) were selected for the shrimp category, and 25 samples were taken from each type of seafish: rupchanda (*Pampus chinensis*), tuna (*Euthynnus affinis*), and loitta (*Harpadon nehereus*). Sampling visits were conducted every two weeks at different retail fish markets throughout the research period. Only fresh and healthy samples (not frozen or previously processed) were collected in this study. Fish with visible signs of disease or injury were excluded from the study. Medium-sized shrimp, crabs, and fish were collected during this study. Two expert veterinarians and two expert microbiologists selected and collected all the samples. After collection, each sample was immediately placed in a sterile zipper bag and kept on ice for transportation to the laboratory.

In the lab, the external surface of the samples underwent cleaning treatment with 70 % (v/v) alcohol. In the case of seafish, each 25 g sample was homogenized with 225 mL of buffered peptone broth (HiMedia, Mumbai, Maharashtra, India) and allowed to incubate overnight at 37 °C [39]. As for shrimp and crabs, each sample's brain, leg, muscle, and intestine were blended, and 25 g of the resulting mixture underwent homogenization and incubation [40].

2.3. Isolation and identification of E. faecalis

After an overnight enrichment, a loopful (10 μL) of the sample was streaked onto an enterococcus agar base plate (HiMedia, Mumbai, Maharashtra, India) and incubated at 37 °C for 24 h. A suspected *E. faecalis* colony, identified by its characteristic morphology, underwent staining and biochemical tests (positive in the Pyrrolidonyl Aminopeptidase test and negative in the catalase test) for additional confirmation of *E. faecalis*. Subsequently, the isolates were preserved in 50 % glycerol, frozen, and subjected to further investigation. The polymerase chain reaction (PCR), using *E. faecalis*-specific primers targeting $ddl_{E.\ faecalis}$ gene (Supplementary Table S1) was performed to identify the presence of *E. faecalis* using extracted genomic DNA of the isolates by boiling method [10,41,42]. The genomic DNA of the isolates was extracted using the following steps: (1) 1 mL portion of the enriched culture was subjected to centrifugation at 5000 rpm for 5 min and the supernatant was discarded; (2) a suspension was prepared by introducing 200 μL of phosphate buffer solution (PBS) and mixed properly with vortex; (3) the suspension was subjected to a 10-min boiling step, followed by a 10 min subsequent cooling period; (4) the suspension was then introduced to another centrifugation with 10,000 rpm for 10 min; and (5) the resulting supernatant containing genomic DNA was collected and stored at a temperature of -20^0C to future analysis.

2.4. Biofilm forming ability of E. faecalis

The qualitative and quantitative assessment of biofilm formation in *E. faecalis* isolates was conducted through the Congo Red (CR) test [43] and the crystal violet microtiter plate (CVMP) technique with 96-well polyester microtiter plates [44]. In the CR test, isolates with dry filamentous crusty black colonies on the plate were classified as strong biofilm producers, those with pink colonies and a dark center as intermediate producers and isolates with smooth pink colonies as non-biofilm producers [45,46]. In the CVMP test, strong biofilm producers were defined as isolates with an optical density (OD_{570} nm value) of $\geq$1, intermediate biofilm producers as isolates with an OD_{570} nm value between <1 and $\geq$ 0.1, and non-biofilm formers as isolates with an OD_{570} nm value of <0.1 [47].

2.5. Detection of virulence genes

A simplex PCR assay was used to detect the presence of virulence-associated genes in *E. faecalis*, including *pil, ace, agg, fsrA, fsrB, fsrC, gelE, sprE,* and *cyl* (Supplementary Table S1). The PCR method employed for the detection of virulence genes was consistent with those previously outlined in section 2.3. Additionally, the PCR-positive controls included genomic DNA from *E. faecalis* that had previously shown positive results for the specific virulence genes. Non-DNA templates were applied as PCR-negative controls, wherein PBS was used in place of genomic DNA.

2.6. Antibiotic susceptibility testing

Following the Clinical and Laboratory Standards Institute guidelines [48], we used the disk diffusion method [49] to evaluate antibiotic susceptibility in *E. faecalis* isolates. A sterile 0.85 % normal saline solution was prepared by suspending 2–3 bacterial colonies and adjusting to 0.5 McFarland standard units. After a 24-h incubation at 37 °C, the bacterial inoculum was spread on Mueller-Hinton agar plates using sterile cotton swabs. Then, selected antibiotics were applied to the agar plates. In this study, we chose 13 widely available antibiotics from ten distinct antibiotic classes. The three antibiotic group's access, watch, and reserve, as defined by the World Health Organization (WHO), were also taken into consideration while choosing an antibiotic (Supplementary Table S2). Multidrug-resistant (MDR) isolates were those that showed resistance to at least three antimicrobial groups [50]. The multiple antibiotic resistance (MAR) indices were determined by using the following formula [51]:

$$\text{MAR index} = \frac{\text{The count of antibiotics to which an isolates showed resistance}}{\text{The total number of antibiotics employed in this study}}$$

Moreover, the beta-lactamase bla_{TEM} gene in *E. faecalis* isolates was detected by a simplex PCR protocol (Supplementary Table S1).

2.7. Statistical analysis

Data from the investigation was inputted into Excel 365 (Microsoft/Office 365, Redmond, WA, USA) and then transferred to SPSS. v.25.0 (IBM, Chicago, IL, USA) and GraphPad Prism.v.8.4.2 (San Diego, CA, USA) for analysis. Descriptive analysis determined *E. faecalis*-associated prevalence, with a binomial 95 % confidence interval (CI_{95}) computed using GraphPad Prism [52]. Chi-square tests identified variations in isolate frequencies and relationships between biofilm formation, virulence genes, and antibiotic resistance. A *P*-value $\leq$0.05 indicated statistical significance. Bivariate analysis in SPSS explored associations between virulence genes and antibiotic resistance observed in *E. faecalis* isolates. A *P*-value less than 0.05 was deemed significant.

3. Results

3.1. Prevalence of E. faecalis in seafood samples

In PCR, 29.3 % (44/150, CI_{95}: 22.6; 37.1) of the samples were contaminated with *E. faecalis,* and there was a statistically significant

variation ($P = 0.04$) among the sample types. Among them, marine fish (36 %, CI_{95}: 20.3; 55.5) had a significantly higher prevalence of *E. faecalis* than shrimp (16 %, CI_{95}: 8.3; 28.5) samples. In addition, *E. faecalis* was found in 36 % (CI_{95}: 26.1; 47.3) of the crab samples (Fig. 1, Supplementary Fig. S3, Supplementary Table S4).

3.2. Prevalence of biofilm-forming E. faecalis

Based on both CR and CVMP tests, 39 (88.6 %, CI_{95}: 76.0; 93.9) out of 44 *E. faecalis* isolates showed biofilm-forming ability. Moreover, the prevalence of intermediate biofilm-forming *E. faecalis* (63.6 %, CI_{95}: 48.9; 76.2) was significantly higher than the prevalence of strong (25 %, CI_{95}: 14.6; 39.4) and non-biofilm-forming (11.4 %, CI_{95}: 4.9; 23.9) *E. faecalis* isolates (Fig. 2; Supplementary Table S5). Interestingly, in terms of samples, crab samples exhibited the highest proportion ($P > 0.05$) of strong biofilm-forming *E. faecalis* at 12 % (3/25, CI_{95}: 4.2; 29.9), followed by marine fish at 8 % (6/75, CI_{95}: 3.7; 16.4) and shrimp at 4 % (2/50, CI_{95}: 0.7; 13.5) (Supplementary Table S3).

3.3. Characteristics of E. faecalis isolates

3.3.1. Virulence profiles

During PCR screening, it was determined that all *E. faecalis* isolates carried a minimum of two out of the nine virulence genes examined. Specifically, twenty-six isolates were identified as having eight out of the nine virulence genes tested (Supplementary Fig. S1, Supplementary Table S3, and Supplementary Figs. S4–S11). The highest prevalence of these virulence genes was observed for *ace* and *pil* (97.7 %, CI_{95}: 88.2; 99.9), followed by *sprE* (95.5 %, CI_{95}: 84.9; 99.2), *gelE* (90.9 %, CI_{95}: 78.8; 96.4), *fsrB* (79.6 %, CI_{95}: 65.5; 88.9), *agg* (70.5 %, CI_{95}: 55.8; 81.8), *fsrA* (68.2 %, CI_{95}: 53.4; 80.0), and *fsrC* (61.4 %, CI_{95}: 46.6; 74.4). However, none of the *E. faecalis* isolates were found to possess the *cyl* virulence gene (Table 1).

The prevalence of the investigated virulence genes (except *cyl*) exhibited a higher trend and significant ($P < 0.05$) variation (except *pil*) in the strong biofilm-forming *E. faecalis* isolates than in intermediate and non-biofilm-forming *E. faecalis* isolates, i.e., *ace* (strong: 100 % vs. intermediate: 100 % vs. non: 80 %), *pil* (100 % vs. 96.3 % vs. 100 %), *sprE* (100 % vs. 100 % vs. 60 %), *gelE* (100 % vs. 96.4 % vs. 40 %), *fsrB* (100 % vs. 82.1 % vs. 20 %), *agg* (100 % vs. 71.4 % vs. 0 %), *fsrA* (90.9 % vs. 71.4 % vs. 0 %), and *fsrC* (90.9 % vs. 60.7 % vs. 0 %) (Table 1).

Moreover, in bivariate analysis, positive and strong significant correlations were observed between the presence of virulence genes *fsrA* and *agg* ($\rho = 0.948$), *fsrC* and *agg* ($\rho = 0.816$), *fsrB* and *agg* ($\rho = 0.783$), *fsrA* and *fsrC* ($\rho = 0.761$), *fsrA* and *fsrB* ($\rho = 0.742$), *gelE* and *sprE* ($\rho = 0.690$), *fsrB* and *fsrC* ($\rho = 0.639$), *fsrB* and *gelE* ($\rho = 0.624$), *agg* and *gelE* ($\rho = 0.488$), *gelE* and *pil* ($\rho = 0.483$), *fsrA* and *gelE* ($\rho = 0.463$), *fsrB* and *sprE* ($\rho = 0.430$), and *fsrC* and *gelE* ($\rho = 0.399$) in *E. faecalis* isolates. Moreover, moderate to low positive and significant correlations were determined between the presence of virulence genes *agg* and *sprE* ($\rho = 0.337$), *fsrA* and *sprE* ($\rho = 0.319$), and *fsrB* and *pil* ($\rho = 0.301$) in *E. faecalis* isolates (Supplementary Table S6).

3.3.2. Antibiotic resistance profiles

Enterococcus faecalis isolates demonstrated resistance to each of the three antibiotic groups classified by the WHO, including access, watch, and reserve categories. Specifically, all tested isolates exhibited resistance to at least two out of the 13 antibiotics tested (Supplementary Fig. S2). Moreover, all the *E. faecalis* isolates were phenotypically resistant to penicillin (100 %, CI_{95}: 91.9; 100), followed by ampicillin and rifampin (86.4 %, CI_{95}: 73.3; 93.6), erythromycin (13.6 %, CI_{95}: 6.4; 26.7), vancomycin, tetracycline, norfloxacin, and linezolid (2.3 %, CI_{95}: 0.1; 11.8) (Table 2 and Supplementary Table S3). Notably, none of the isolates displayed resistance to ciprofloxacin, levofloxacin, nitrofurantoin, fosfomycin, and chloramphenicol. In addition, 15.9 % (7/44, CI_{95}: 7.9; 29.4) of the *E faecalis* isolates exhibited phenotypic multidrug resistance, and 72.7 % (32/44, CI_{95}: 58.2; 83.7) of the isolates had more than 0.2 MAR index (Supplementary Table S3). The presence of the beta-lactamase gene bla_{TEM} was detected in 61.4 % (27/44, CI_{95}: 46.6;

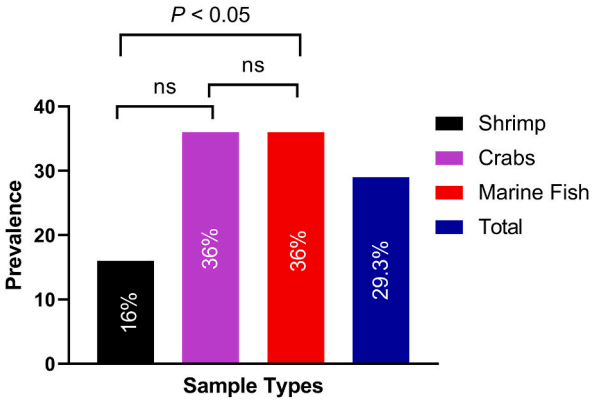

Fig. 1. Prevalence of *Enterococcus faecalis* isolated from different seafoods in Bangladesh; ns = not significantly different ($P > 0.05$).

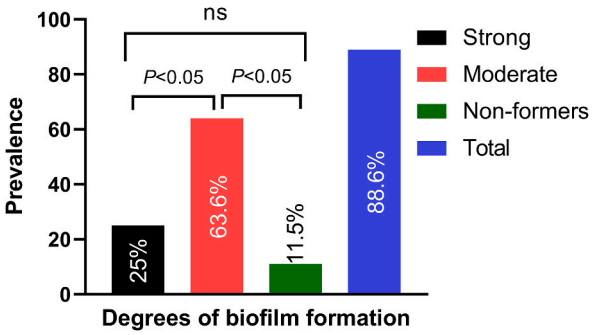

Fig. 2. Prevalence of biofilm-forming *Enterococcus faecalis* isolates from seafood samples in Bangladesh; ns = not significantly different ($P > 0.05$).

Table 1
Association in the detection of virulence genes and determination of biofilm formation in *E. faecalis* (n = 44) isolated from raw seafood in Bangladesh.

Virulence Genes	Virulence in Different Degrees of Biofilm Formation			Total no. of Positive Isolates (%) [95 % CI]	*P*-value
	No. (%) of Strong Biofilm Former (n = 11)	No. (%) of Intermediate Biofilm Former (n = 28)	No. (%) of Non-Biofilm Former (n = 5)		
agg	11 (100^{a})	20 (71.4^{a})	0 (0^{b})	31 (70.5) [55.8–81.8]	<0.001
fsrA	10 (90.9^{a})	20 (71.4^{a})	0 (0^{b})	30 (68.2) [53.4–80.0]	0.001
fsrB	11 (100^{a})	23 (82.1^{a})	1 (20^{b})	35 (79.6) [65.5–88.9]	0.001
fsrC	10 (90.9^{a})	17 (60.7^{a})	0 (20^{b})	27 (61.4) [46.6–74.4]	0.002
gelE	11 (100^{a})	27 (96.4^{a})	2 (40^{b})	40 (90.9) [78.8–96.4]	<0.001
sprE	11 (100^{a,b})	28 (100^{b})	3 (60^{a})	42 (95.5) [84.9–99.2]	<0.001
ace	11 (100^{a,b})	28 (100^{b})	4 (80^{a})	43 (97.7) [88.2–99.9]	0.018
pil	11 (100^{a})	27 (96.4^{a})	5 (100^{a})	43 (97.7) [88.2–99.9]	0.747
cyl	0 (0^{a})	0 (0^{a})	0 (0^{a})	0 (0) [0.0–8.0]	NA

Here, values with different superscripts differ significantly ($p < 0.05$) within the variable under assessment, CI = confidence interval, NA = not applied.

74.3) of the *E. faecalis* isolates (Table 2, Supplementary Fig. S12, and Supplementary Table S3).

Interestingly, *E. faecalis* isolates with strong biofilm-forming capabilities exhibited elevated tendencies of resistance to a variety of antibiotics and the resistance gene, including ampicillin (strong: 100 % vs. intermediate: 85.7 % vs. non: 60 %), rifampin (100 % vs. 89.3 % vs. 40 %), erythromycin (45.5 vs. 3.6 % vs. 0 %), vancomycin, tetracycline, norfloxacin, linezolid (9.1 % vs. 0 % vs 0 %), and the resistance bla_{TEM} gene (90.9 % vs. 57.1 % vs 20 %) (Table 2). Moreover, a statistically significant correlation ($P < 0.05$) was identified between the degrees of biofilm formation and the resistance profiles of *E. faecalis* isolates to erythromycin, rifampin, and the presence of the beta-lactamase bla_{TEM} gene (Table 2).

Table 2
Association of antibiotic resistance patterns and biofilm formation in *E. faecalis* strains (n = 44) detected in raw seafood in Bangladesh.

Categories	Antibiotics	Antibiotic Resistance in Different Degrees of Biofilm Formation			Total no. of Positive Isolates (%) [95 % CI]	*P*-value
		No. (%) of Strong Biofilm Former (n = 11)	No. (%) of Intermediate Biofilm Former (n = 28)	No. (%) of Non-Biofilm Former (n = 5)		
Phenotypic	AMP	11 (100^{a})	24 (85.7^{a})	3 (60^{a})	38 (86.4) [73.3–93.6]	0.095
	VA	1 (9.1^{a})	0 (0^{a})	0 (0^{a})	1 (2.3) [0.1–11.8]	0.215
	E	5 (45.5^{a})	1 (3.6^{b})	0 (0^{a,b})	6 (13.6) [6.4–26.7]	0.002
	TE	1 (9.1^{a})	0 (0^{a})	0 (0^{a})	1 (2.3) [0.1–11.8]	0.215
	NOR	1 (9.1^{a})	0 (0^{a})	0 (0^{a})	1 (2.3) [0.1–11.8]	0.215
	LZD	1 (9.1^{a})	0 (0^{a})	0 (0^{a})	1 (2.3) [0.1–11.8]	0.215
	RD	11 (100^{a})	25 (89.3^{a})	2 (40^{b})	38 (86.4) [73.3–93.6]	0.004
	CIP	0 (0^{a})	0 (0^{a})	0 (0^{a})	0 (0) [0.0–8.0]	NA
	LEV	0 (0^{a})	0 (0^{a})	0 (0^{a})	0 (0) [0.0–8.0]	NA
	FOS	0 (0^{a})	0 (0^{a})	0 (0^{a})	0 (0) [0.0–8.0]	NA
	P	11 (100^{a})	28 (100^{a})	5 (100^{a})	44 (100) [91.9–100]	NA
	NIT	0 (0^{a})	0 (0^{a})	0 (0^{a})	0 (0) [0.0–8.0]	NA
	C	0 (0^{a})	0 (0^{a})	0 (0^{a})	0 (0) [0.0–8.0]	NA
Genotypic	bla_{TEM}	10 (90.9^{a})	16 (57.1^{a,b})	1 (20^{b})	27 (61.4) [46.6–74.3]	0.02

Here, values with different superscripts differ significantly ($p < 0.05$) within the variable under assessment; CIP = ciprofloxacin, TE = tetracycline, LEV = levofloxacin, FOS = fosfomycin, RD = rifampin, P = penicillin, LZD = linezolid, NOR = norfloxacin, NIT = nitrofurantoin, AMP = ampicillin, C = chloramphenicol, VA = vancomycin, E = erythromycin, CI = confidence interval, NA = not applied.

Additionally, in the bivariate analysis, positive significant correlations were detected between the phenotypic resistance of *E. faecalis* isolates to pairs of antibiotics, specifically linezolid and vancomycin ($\rho = 1.000$), erythromycin and tetracycline ($\rho = 0.384$), and erythromycin and norfloxacin ($\rho = 0.384$) (Supplementary Table S7).

4. Discussion

4.1. Enterococcus faecalis in seafood samples

In the present study, *E. faecalis* were PCR-positive in 29.3 % of seafood samples, contaminating all the examined sample types, where a comparably higher rate was shown in marine fish and crabs than in shrimp samples. The higher occurrence of *E. faecalis* in fish samples could be linked to suboptimal hygiene conditions and the potential for cross-contamination between different fish samples. This underscores the importance of exercising caution when determining the origin of isolated *E. faecalis*. The samples under examination were procured from retail sources, and the heightened prevalence of *E. faecalis* may indicate the possibility of fecal contamination from both human and animal sources within the aquaculture environment. Alternatively, these bacteria might have been acquired during processing, considering that they are not typically part of the natural bacterial flora associated with fish, shrimp, and crabs.

Previously, similar findings were recorded by Hammad et al. [39] in Japan and Igbinosa and Beshiru [53] in Nigeria, detecting *E. faecalis* in 28.8 % and 32.2 % of seafood samples, respectively. However, compared to our findings, a higher prevalence of *E. faecalis* was recorded in seafood samples, e.g., 87 % by Ellis-Iversen et al. [54] in Denmark, 70.2 % by Naas et al. [55] in Lybia, 59 % by Boss et al. [56] in Switzerland, 47.7 % by Ben Said et al. [57] in Tunisia, and 44.3 % by Araújo et al. [58] in Brazil. In contrast, a previous study determined a lower rate of *E. faecalis* in seafood samples in comparison with our study, detecting 20.2 % by Noroozi et al. [27] in Iran. The differences observed in the occurrence of *E. faecalis* isolates within seafood samples could be attributed to a multitude of factors. These may include the geographical settings where the studies were conducted, encompassing diverse environmental conditions that could influence microbial populations. Furthermore, variations in the types and quantities of seafood samples collected, as well as the microbial loads present within these samples or their respective locales, might also contribute to the observed discrepancies.

4.2. Biofilm-forming capabilities of isolated E. faecalis

In this study, 88.6 % of *E. faecalis* isolates were biofilm formers, where strong and intermediate biofilm-forming abilities were observed in 25 % and 63.64 % of the isolates. Previously, Igbinosa and Beshiru [53] and Chajecka-Wierzchowska et al. [59] also determined biofilm-forming abilities in 82.4 % and 45.5 % of *E. faecalis* isolates, respectively. The presence of *E. faecalis*, which is capable of forming biofilms in seafood, poses significant public health risks, as biofilm development facilitates the emergence of AMR and virulence traits within bacterial pathogens. Moreover, our results indicate the presence of biofilm-forming *E. faecalis* in seafood samples, potentially attributed to inadequate post-processing sanitation measures. It's crucial to recognize that these biofilm-producing bacteria have the capability to migrate considerable distances along the production line, thereby increasing the likelihood of equipment malfunctions, food spoilage, and posing health hazards if they contaminate batches intended for consumers [60].

4.3. Virulence factors in isolated E. faecalis

In this study, at least two tested virulence genes were detected in all the *E. faecalis* isolates, where eight out of nine virulence genes were found to be positive in 59.1 % of the isolates. The majority of *E. faecalis* isolates in this study were found to be positive for the presence of virulence genes *pil* and *ace*. The *pil* and *ace* are two significant virulence factors that are responsible for the adhesion and colonization of the host due to their associated products [13]. Furthermore, our current study revealed that *E. faecalis* isolates possessed additional virulence genes, including *fsrA, fsrB, fsrC, sprE*, and *gelE*, which are associated with the formation and strength of biofilms in *E. faecalis* [13,15]. The notable abundance of virulence genes identified within *E. faecalis* isolates from seafood samples raises significant concerns for human health. Our results suggest that seafood could potentially act as a significant vehicle for disseminating these virulent *E. faecalis* strains to both humans and the surrounding environment. Nevertheless, further investigation is warranted to understand this phenomenon fully. Previously, like our study, several studies also detected various virulence genes in *E. faecalis* isolated from seafood samples [27,39,53,56–58]. In this study, no isolates harbored the virulence gene *cyl*. However, previous studies detected *cyl* from *E. faecalis* isolated from seafood samples, e.g., 33.9 % by Noroozi et al. [27], 10.2 % by Igbinosa & Beshiru [53], 8.5 % by Araújo et al. [58], and 7.3 % by Ahmed et al. [61].

A noteworthy increase in the prevalence of virulence genes was observed in *E. faecalis* isolates, particularly those exhibiting strong and/or moderate biofilm formation. This finding suggests a direct correlation, indicating that as the degree of biofilm formation intensifies in *E. faecalis* isolates, so does their potential to instigate infections. A few previous studies also found similar correlations between the presence of virulence genes and the degree of biofilm formation in bacterial isolates [62–64]. This association indicates that the isolated *E. faecalis* strains are capable of forming biofilms. Nevertheless, more in-depth research is imperative to elucidate the precise relationship between the biofilm-forming capability of enterococci isolates and the manifestation of their virulence genes. Furthermore, other virulence determinants like *asa*, *efa*, *esp*, and *ebp*, though not investigated in this study, could potentially play a role in the strong biofilm-forming ability exhibited by the enterococcal isolates.

4.4. Antimicrobial resistance profiles of E. faecalis

In the present study, the isolated *E. faecalis* were found to have greater rates of resistance to penicillin (100 %), ampicillin (86.36 %), and rifampin (86.36 %) compared to other antibiotics used, i.e., erythromycin (13.7 %), tetracycline, vancomycin, norfloxacin, and linezolid (2.3 %). Moreover, all the isolates showed phenotypic resistance to ≥2 antimicrobial agents, where 15.9 % of the isolates were MDR and 72.7 % of the isolates had ≥0.2 MAR index. As per Krumperman [51], isolates exhibiting a MAR index ≥0.2 were believed to originate from environments posing a heightened risk of contamination, typically associated with frequent antibiotic usage. The likely cause of the high antimicrobial resistance rate is the irregular and unauthorized prescription of such agents. Based on the observation that certain isolates exhibited high resistance to antimicrobial agents typically used to treat human infections, it can be inferred that these isolates may have originated from infected individuals who work in seafood sales and processing centers [65]. The existence of antibiotic-resistant bacteria within seafood raises concerns for public health, as they may serve as conduits for the dissemination of resistance characteristics across the food chain to other bacteria of clinical significance to humans. It is plausible that antibiotic-resistant fecal bacteria, originating from various sources like domestic sewage or animal and fish farming, could transmit their resistance traits to native fish microbiota upon discharge into the sea [10]. This transmission could catalyze the proliferation and prevalence of antibiotic resistance within the marine ecosystem. *E. faecalis* strains isolated from food samples exhibited varying levels of AMR against a range of antimicrobial agents, as documented in studies conducted across diverse regions, including Iran [27], South Korea [66] Poland [67], Turkey [68] Switzerland [56] Africa [69], and Slovakia [70]. Bacteria capable of forming biofilms demonstrated increased resistance levels in comparison to those lacking this ability [71]. Consistent with this statement, *E. faecalis* isolates displaying strong biofilm-forming capabilities exhibited heightened resistance to the antibiotics tested in this study. This suggests a plausible correlation between the biofilm-forming potential of *E. faecalis* isolates and their antimicrobial resistance.

5. Conclusions

Our study of the molecular ecology and population dynamics of isolated strains emphasizes the significance of seafood samples as a repository for *E. faecalis* harboring both resistance and virulence genes. With certain strains of *E. faecalis* exhibiting both antibiotic resistance and virulence markers, it becomes crucial to recognize seafood samples as potential reservoirs of these bacteria and antibiotic resistance. Moreover, the results of this study underscore the urgent need for rigorous hygiene practices during the processing, packaging, and storage of seafood in Bangladesh. Additionally, this research advocates for proactive measures to promote responsible antimicrobial usage across different sectors, including in the production of food animals.

CRediT authorship contribution statement

Md Ashek Ullah: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Md Saiful Islam:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Farhana Binte Ferdous:** Investigation. **Md Liton Rana:** Methodology, Investigation. **Jayedul Hassan:** Writing – review & editing, Supervision. **Md Tanvir Rahman:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Informed consent statement

Not applicable.

Data availability statement

All the data are available in the manuscript and Supplementary Material File.

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Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39294.

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