Multi-Antibiotic Resistant *Citrobacter freundii* in Eggs: A Silent Public Health Threat

Jinia Afroz[1], Md. Al Masud[1], Esrat Jahan[1], Ajoy Chowdhury[1], Md. Fakruddin[2], Md. Asaduzzaman Shishir[1,3*]

Abstract

Background: Improper handling of poultry, particularly in developing countries like Bangladesh, can lead to foodborne illnesses. *Citrobacter freundii*, a Gram-negative bacteria, is a silent emerging threat causing diseases and spreading antibiotic resistance. This study aimed to identify the multidrug-resistant (MDR) *Citrobacter freundii* from poultry egg samples through biochemical and molecular characterization. **Materials and Methods:** A total of 50 poultry egg samples (both outer and inner) were collected from different farms located in Dhaka, Bangladesh within six months. Isolation of pathogens with selective bacteriological media such as McConkey agar, Xylose Lysine Deoxycholate agar, Salmonella-Shigella agar, and specific biochemical tests were performed to identify the bacterial isolates. The antibiotic sensitivity pattern of the isolates was determined by the disk diffusion method. Multidrug-resistant isolates were further identified by 16S rRNA gene sequencing and BLASTn analysis. **Results:** Out of 50 samples, 60% (30) were found positive for Gram-negative Enterobacteriaceae. Among them, 20% of the samples were found to be contaminated with *Citrobacter* spp, followed by *Salmonella enterica* (5) 16.67%, and 13.33% with *Yokenella* (4) and *Serratia* (4) each. They were highly resistant to erythromycin (90%), Amoxicillin (77%), Cefixime (57%), Chloramphenicol (50%), and Azithromycin (43%). On the contrary, they were sensitive against imipenem (93%), Gentamycin (70%), and Ciprofloxacin (60%). Among all *Citrobacter* isolates, a randomly chosen multidrug-resistant isolate was identified based on its 16S rRNA gene sequence with the highest similarity (100%) to *Citrobacter freundii* strain GMU8049.**Conclusion:** Poultry eggs can be a source of transmission of multidrug-resistant *Citrobacter freundii* from animal to human. It could be a serious health concern for developing countries since very little effort has been put into this issue.

Keywords: *Citrobacter freundii*, Human health, Poultry eggs, Multidrug resistance.

Significance | Finding Silent Public Health Threats

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1. Introduction

With approximately 90 billion metric tonnes of annual meat production, chicken is the most widely farmed species and one of the most significant sources of protein in the world (Machuve et al., 2022). Poultry products, notably eggs, and egg products are healthy food items that constitute an essential part of people's daily diets all over the world (Stepien-Pysniak, 2010). There are many hygienic grades of eggs available, and if not handled properly, the poorer qualities can result in foodborne illnesses when consumed. As a result, citizens of developing and underdeveloped countries experience regular health problems like

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diarrhoeal illnesses, enteric fever, etc., and similar instances are also sometimes reported in developed countries (Akbar & Anal, 2011).

Eggs produced in poultry farms may be contaminated by pathogens that are transmitted both horizontally and vertically. In the transovarial pathway, vertical transmission takes place during bacterial shedding from the hen's infected reproductive organs. This may happen before the shell covers the eggs and thus directly contaminates the yolk, albumen, and membranes (Messens et al., 2005). The egg's passage through the highly contaminated cloaca area at the time of laying causes horizontal contamination, which allows pathogens to enter the eggs' shells (De Reu et al., 2008).

The FAO reports that during the past 25 years, Bangladesh has experienced a sharp increase in the production of poultry meat and eggs. Production of poultry meat has climbed from 660 metric tonnes in 1990 to 6.2 million metric tonnes in 2016; within the same period, production of eggs has increased by 11,912.4 million (M. S. Rahman et al., 2017). Unfortunately, there was an uneven distribution of information regarding hygiene understanding and practice. The likelihood of antibiotic resistance in Bangladesh has also increased due to the ease with which all antibiotics may be obtained, as well as the widespread abuse of antibiotics against bacterial, fungal, viral, and parasitic illnesses in the chicken sector.

Poultry frequently contains Enterobacteriaceae (Projahn et al., 2018), *E. coli* (Chousalkar et al., 2010), *Salmonella* spp. (Gantois et al., 2009), and *Klebsiella* spp. (Kowalczyk et al., 2022) are the most prevalent Enterobacteriaceae detected on eggs. *Citrobacter freundii*, an important member of the Enterobacteriaceae family, can infect eggs; however, information on this topic is limited. In addition to being a commensal resident of both human and animal intestinal tracts, *Citrobacter freundii* is a gram-negative, coccobacilli-shaped, facultatively anaerobic motile (due to flagellar movement) bacteria (Liu et al., 2018). It can also cause diarrhea and other infections in humans, such as urinary tract infections, pneumonia, and, in rare cases, meningitis and intracranial abscesses (Bai et al., 2012; Liu et al., 2017; Plakkal et al., 2013). Food-borne illnesses in animals are brought on by *Citrobacter freundii*, which is found in the gastrointestinal system (M. Fakruddin et al., 2014).

To prevent bacterial infections in chickens, antibiotics are frequently employed (Mehdi et al., 2018). Antibiotic use during therapy led to bacterial resistance, which became a hazard to worldwide public health (Prestinaci et al., 2015). When bacteria's sensitivity to antibiotics changes and they gain the ability to resist the drugs, antibiotic resistance occurs naturally; however, it has been exacerbated by misuse and abuse of the drugs (Reygaert, 2018). Antibiotic resistance of *C. freundii* has increased worldwide, and some strains were reported to harbor extended-spectrum β-lactamase (ESBL)(Park et al., 2005) and plasmid-mediated quinolone resistance (PMQR) determinants (Shao et al., 2011).

Like other Enterobacteriaceae, *Citrobacter* sp. is frequently transmitted via the fecal-oral route. It is a zoonotic pathogen that can be transferred to people from chicken sources while handling eggs, processing cooked or raw meat, and handling carcasses in the slaughterhouse (Nandi et al., 2013). *Citrobacter* spp. are low-virulent bacteria that can survive in host populations for extended periods and accumulate antimicrobial resistance (AMR) determinants, which can increase their virulence (Igbinosa et al., 2018). Shrestha et al. found a prevalence of 26.1% for multidrug-resistant and extended-spectrum β-lactamase (ESBL)-producing *Citrobacter* spp., while Kanamori et al. reported a prevalence of 19.3% for ESBL-producing *Citrobacter* spp. (Kanamori et al., 2011; Shrestha et al., 2017).

Due to the overwhelming use of antibiotics for medical and veterinary purposes (Abony et al., 2018; Uddin et al., 2014; White et al., 2000), as well as the domestic and agricultural use of pesticides and related compounds (Balagué & Véscovi, 2001), significant antibiotic contamination of the natural environment and subsequent development of resistance to these agents in communities have occurred in recent years. Therefore, a growing proportion of pathogenic bacteria that are resistant to one or more antibiotics is a worry for global public health, especially in developing countries (Fair & Tor, 2014).

There has been little research on *Citrobacter* spp., their antibiotic resistance, and the probable threat to the public health in Bangladesh. The primary goal of this investigation is to recognize and ascertain the prevalence of *Citrobacter* spp. in chicken eggs and the pattern of antibiotic resistance of the isolates obtained from various locations in Dhaka, Bangladesh.

2. Methods and materials

2.1. Study area and collection of samples

The egg samples were collected fom different local markets and farms in Dhaka, Bangladesh. This study was carried out from July 2022 to December 2022 at the Department of Microbiology, Primeasia University. A total of 50 poultry egg samples were collected following aseptic technique using sterile zipped bags, gloves, etc. During the collection of egg samples, all precautions were maintained to minimize cross-contamination, and all samples were transported directly to the laboratory and analyzed.

2.2. Enrichment and isolation of microorganisms

For the isolation of the bacterial spp., both the outer shell surface and inner portion of the poultry egg sample were used. Isolation was done by pre-enrichment followed by selective enrichment and selective plating techniques. Following this, buffered peptone water (BPW), tetrathionate broth (TT), MacConkey agar (Mac), Xylose-Lysine Deoxycholate (XLD) agar, and Salmonella-Shigella

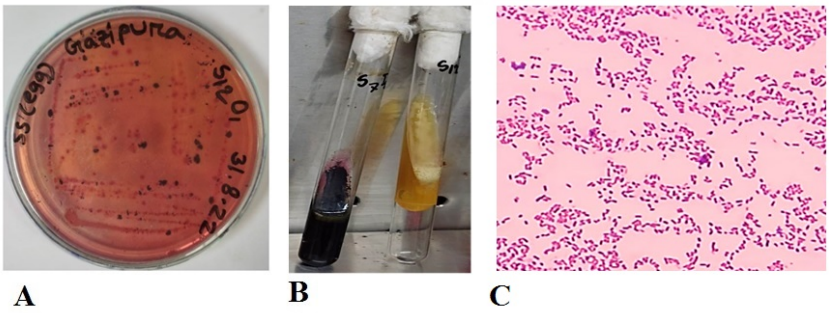

Figure 1. A. Isolates exhibiting their characteristics on selective media, **B.** H_2S gas produced in TSI agar test, **C.** Microscopic observation of the isolates revealed the presence of gram-negative rod-shaped bacilli.

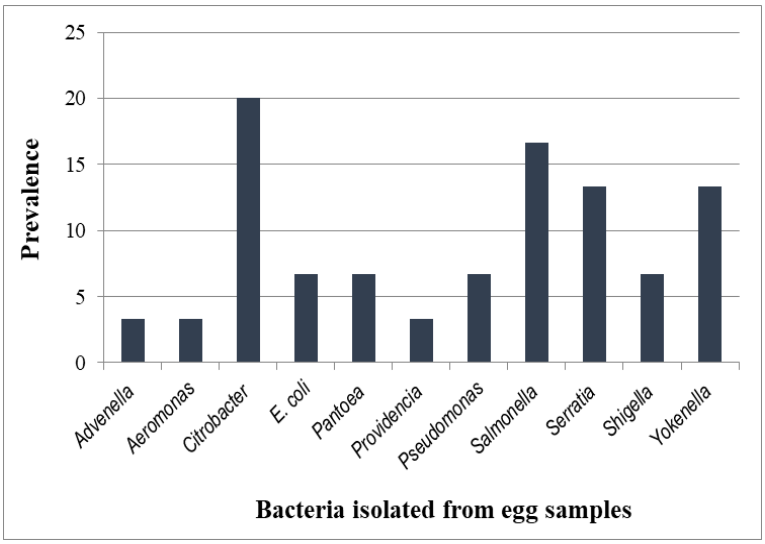

Figure 2. Prevalence of bacterial pathogens in the egg samples.

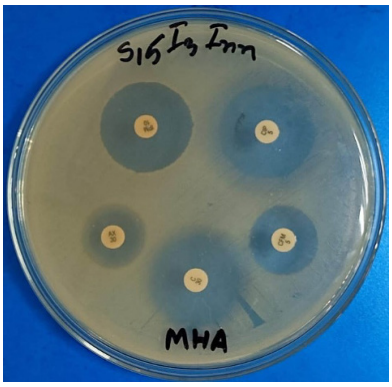

Figure 3. Zone of inhibition on a Muller-Hinton agar (MHA) plate

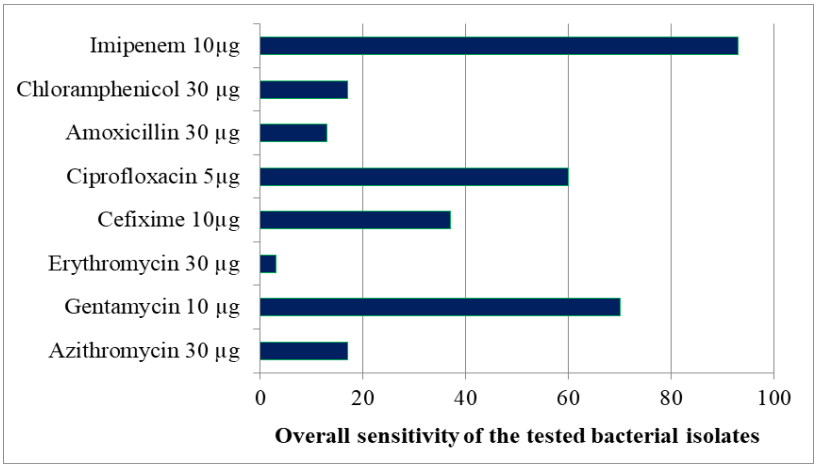

Figure 4. Graphical presentation of the overall sensitivity of the antibiotics used against the bacterial isolates.

Table 1. Biochemical properties of the isolates

Isolates	Oxidase	Glucose fermentation	Lactose Fermentation	Indole Production	Methyl Red (MR)	Voges Proskaur (VP)	Citrate Utilization	H_2S production	Gas Production	Presumptive Identity
S1	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	*Serratia fonticola*
S5	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	*Shigella*
S9	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	*Pseudomonas*
S7I1	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	*Aeromonas hydrophila*
S7I2	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	*Citrobacter freundii*
S8I1	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Pantoea dispersa*
S8I2	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	*Escherichia coli*
S9	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	*Pseudomonas*
S10	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	*Citrobacter freundii*
S11I1	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	*Pantoea dispersa*
S11I2	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	*Serratia rubidaea*
S12I1	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	*Serratia fonticola*
S12I2	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	*Advenella faeciporci*
S11I1 (I)	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	*Citrobacter freundii*
S11I2 (I)	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	*Citrobacter freundii*
S12 (I)	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	*Escherichia coli*
S15I1 (I)	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	*Shigella sonnei*
S15I2 (I)	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	*Salmonella enterica*
S16I1	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	*Serratia fonticola*
S16I2	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	*Salmonella enterica*
S17	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Yokenella regensburgei*
S18	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	*Salmonella enterica*
S21I1	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Yokenella regensburgei*
S21I2	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	*Salmonella enterica*
S23	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Yokenella regensburgei*
S24	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	*Citrobacter freundii*
S25I1	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*Citrobacter diversus*
S25I2	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Yokenella regensburgei*
S27	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	*Providencia alcalifaciens*
S28	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	*Salmonella enterica*

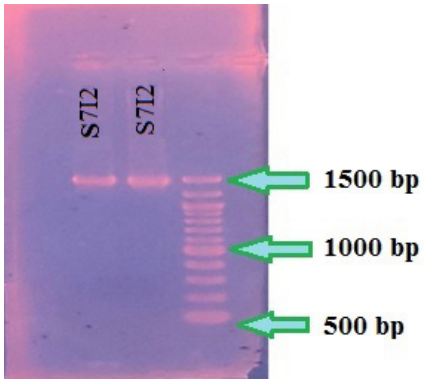

Figure 5. Agarose gel electrophoresis (1.5%) of PCR products after amplification of 16S rRNA gene for molecular identification. Lane M-1 Kb DNA ladder (); Lane 1-2: PCR products. The band was observed at 1500 bp.

Table 2. Antibiotic sensitivity analysis of the bacterial isolates.

Isolates ID	Azithromycin 30 ug	Gentamycin 10 µg	Erythromycin 30 ug	Cefixime 10µg	Ciprofloxacin 5ug	Amoxicillin 30 µg	Chloramphenicol 30 ug	Imipenem 10µg	Presumptive identity	Sensitivity (%)	Intermediate (%)	Resistance (%)
S1	I	S	R	S	S	S	I	S	*Serratia fonticola*	62.5	25	12.5
S5	S	S	S	S	S	I	R	S	*Shigella*	75	12.5	12.5
S9	S	S	I	I	S	S	R	S	*Pseudomonas*	62.5	25	12.5
S7I1	R	S	R	R	S	R	R	S	*Aeromonas hydrophila*	37.5	0	62.5
S7I2	I	I	R	S	S	R	R	S	*Citrobacter freundii*	37.5	25	37.5
S8I1	S	S	R	R	S	I	S	S	*Pantoea dispersa*	62.5	12.5	25
S8I2	S	R	I	R	S	I	R	S	*Escherichia coli*	37.5	25	37.5
S10	R	S	R	I	S	S	R	S	*Pseudomonas*	50	12.5	37.5
S11I1	I	S	R	S	S	R	R	S	*Citrobacter freundii*	50	12.5	37.5
S11I2	R	S	R	S	S	S	R	S	*Pantoea dispersa*	62.5	0	37.5
S12I1	R	I	R	S	S	R	S	S	*Serratia rubidaea*	50	12.5	37.5
S12I2	I	I	R	S	S	R	R	S	*Serratia fonticola*	37.5	25	37.5
S11I1 INNER	R	I	R	S	S	R	I	S	*Advenella faeciporci*	37.5	25	37.5
S11I2 INNER	R	S	R	S	S	R	R	S	*Citrobacter freundii*	50	0	50
S12 INNER	I	I	R	S	S	R	I	S	*Citrobacter freundii*	37.5	37.5	25
S15I1 INNER	R	S	R	S	S	R	R	S	*Escherichia coli*	50	0	50
S15I2 INNER	R	S	R	R	S	R	R	S	*Shigella sonnei*	37.5	0	62.5
S16I1	R	I	R	R	R	R	I	S	*Salmonella enterica*	12.5	25	62.5
S16I2	I	S	R	R	R	R	I	S	*Serratia fonticola*	25	25	50
S17	S	S	R	R	S	R	S	S	*Salmonella enterica*	62.5	0	37.5
S18	I	S	R	R	I	R	S	S	*Yokenella regensburgei*	37.5	25	37.5
S21I1	R	I	R	R	R	R	R	I	*Salmonella enterica*	0	25	75
S21I2	I	S	R	R	R	R	I	S	*Yokenella regensburgei*	25	25	50
S22	I	S	R	R	R	R	S	S	*Salmonella enterica*	37.5	12.5	50
S23	I	S	R	R	R	R	I	S	*Yokenella regensburgei*	25	25	50
S24	R	I	R	R	R	R	I	I	*Citrobacter freundii*	0	37.5	62.5
S25I1	I	S	R	R	R	R	I	S	*Citrobacter diversus*	25	25	50
S25I2	I	S	R	R	R	R	I	S	*Yokenella regensburgei*	25	25	50
S27	R	S	R	R	R	R	R	S	*Providencia alcalifaciens*	25	0	75
S28	R	S	R	R	R	R	R	S	*Salmonella enterica*	25	0	75
Overall Sensitivity (%)	**17**	**70**	**3**	**37**	**60**	**13**	**17**	**93**				

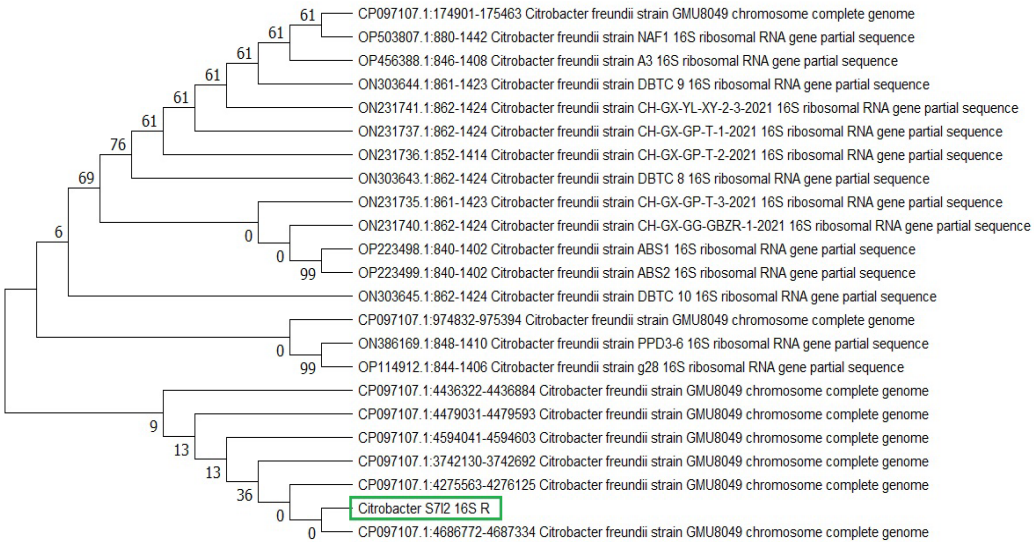

Figure 6. Phylogenetic tree of the A3 strain of *Citrobacter freundii* derived from maximum likelihood analysis of the 16S rRNA gene.

(SS) agar media were used. The outer shell surface of the egg was swabbed with a sterile cotton swab stick and incubated into BPW for pre-enrichment (Fair & Tor, 2014). For a sampling of the inner portion of the egg, the outer shell surface was washed with 70% alcohol, air-dried, and then cracked with a sterile knife. Egg yolk and egg white were thoroughly homogenized and pre-enriched in BPW for 24 hours (Monzur et al., 2012). After pre-enrichment, for selective enrichment purposes, tetrathionate broth was used, and then a loopful from each of the selective enrichment broths was streaked onto Mac agar (HiMedia), XLD agar (HiMedia) and SS agar (HiMedia) and the plates were incubated at 37 °C for 24 hours.

2.3 Analysis of morphological and biochemical properties

Colonies obtained on Mac agar, XLD agar and SS agar were selected as the member of enterobacteriaceae family (Hashim & AlKhafaji, 2018; Sai et al., 2023). Bacterial smears were prepared from the colonies for microscopic observation and the colonies were used for biochemical tests. The biochemical tests performed were Oxidase, glucose fermentation, lactose fermentation, triple sugar iron agar (TSI) test, catalase, IMVIC (Indole production test, Methyl red, Voges Proskauer, and Citrate utilization test), etc.

2.4 Antimicrobial susceptibility test

The bacterial isolates obtained from the egg samples were checked for their antimicrobial susceptibility. According to the recommendations of the Clinical and Laboratory Standards Institute (Weinstein & Lewis, 2020), antimicrobial susceptibility was assessed using the disc diffusion method on Mueller-Hinton agar (HI Media, India). The following eight antibiotics were tested: Azithromycin (30 µg), Gentamicin (10 µg), Erythromycin (30 µg), Cefixime (10 µg), Ciprofloxacin (5 µg), Amoxicillin (30 µg), Chloramphenicol (30 µg), and Imipenem (10 µg). The diameters of the zones of inhibition were recorded and results were interpreted according to CLSI guidelines (FR, 2012).

2.5 Molecular identification of the MDR bacteria

DNA template was prepared from the multidrug resistant bacteria following boil template preparation method (Aktar et al., 2021; Shishir et al., 2015). The PCR was performed to amplify the 16S rRNA gene of the bacterial isolate. Universal primer pairs 27F: AGA GTT TGA TCM TGG CTC AG and 1492 R: CGG TTA CCT TGT TAC GAC TT were used in this experiment. Master Mix (10 µl), template DNA (25–50 ng/µl) (1 µl), forward primer (10 µM/µl) (1 µl), reverse primer (10 µM/µl) (1 µl), and PCR grade water (7 µl) all were included in the reaction mixture (20 µl). The PCR amplification was done with an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 90 s. The final extension was carried out at 72 °C for 5 minutes. PCR products were then analyzed through agarose gel (1.5% agarose, Promega, USA) electrophoresis (70 Volts for 60 minutes) with ethidium bromide as a DNA staining agent and visualized in an Alpha Imager HP gel documentation system. The amplified product was purified and subjected to sequencing for phylogenetic characterization. The data were analyzed by using Finch TV software, version 1.4.0 (www.geospiza.com) (Treves, 2010). The sequences were compared to the reference 16S rRNA sequences at the National Center for Biotechnology Information (NCBI) database by using the Basic Local Alignment Search Tool (BLAST) algorithm. The phylogenetic tree was constructed based on the nucleotide sequences by using Molecular Evolutionary Genetic Analysis (MEGA-11) software (M. D. Fakruddin et al., 2015; Kumar et al., 2016; S. Rahman et al., 2022).

3. Results

Out of 50 egg samples, 60% (30) were found to harbor Gram-negative bacteria since growth was observed on MacConkey agar plate. Following oxidase test, four isolates were found to be positive and remaining twenty six (26) belonged to the Enterobacteriaceae family. In combination with selective bacteriological media, biochemical tests (Table 1) and microscopic observation (Fig 1), the bacteria were presumptively identified. Among them (n=30), 20% (6) of the samples were found to be contaminated with *Citrobacter freundii*, 16.67% (5) with *Salmonella*, 13.33% (n=4) with *Yokenella* and *Serratia* each, etc. (Fig. 2). The biochemical properties of the isolates were used for their presumptive identification and it was performed in combination with Bergey's manual and ABIS online.

Among those contaminated egg samples, bacterial presence on the eggshells was in 84% of cases whereas it was in the egg yolk and egg white portions in 16% of cases.

Only four (4) out of thirty (30) isolates were oxidase negative. Hence, twenty six (26) were grouped into the Enterobacteriaceae family and checked for other properties such as fermentation ability of glucose and lactose, gas production, H_2S production, IMViC tests, etc. All twenty six isolates belonging to Enterobacteriaceae family were Methyl Red positive and Voges Proskauer negative. However, rest of the properties were variable among the isolates (Table 1).

The antibiotic susceptibility of the isolates was determined against eight commonly used antibiotics belonging to different genera (Fig. 3). It was observed that the maximum isolates were resistant against Erythromycin (90%) followed by Amoxicillin (77%), Cefixime (57%), Chloramphenicol (50%), and Azithromycin (43%) (Table 2).

Imipenem was the most effective antibiotic with 93% sensitivity followed by Gentamycin (70%), Ciprofloxacin (60%) and Cefixime (37%). Erythromycin and Amoxicillin were the least effective with 3% and 13% sensitivity respectively whereas Azithromycin and Chloramphenicol both demonstrated only 17% sensitivity (Fig. 4).

Although no resistance was observed against Imipenem, two bacterial isolates such as S21I1 and S24 (*Salmonella enterica* and *Citrobacter freundii* respectively) showed intermediate response.

Gentamycin resistant isolate was S8I2 which was biochemically presumed as *Escherichia coli* and intermediate response was observed from *Serratia, Advenella faeciporci, Salmonella,* and *Citrobacter*.

When the isolates were closely observed, it was found that S21I1, S27, and S28 were most resistance (75%) against the antibiotics checked in this study followed by S7I1, S15I2 (Inner), S16I1 and S24 (62.5% resistance). These bacteria were *Salmonella enterica*, *Providencia alcalifaciens*, *Citrobacter freundii*, *Shigella sonnei* and *Aeromonas hydrophilla.*

Citrobacter isolates were found to be dominant among others and their overall antibiotic resistance was 43.75%, i.e., the isolates were resistant to almost half of the antibiotics used. Among them, one was randomly chosen and identified based on their 16S rRNA gene sequences. PCR products of Ca. 1500bp were obtained after the amplification of the gene (Fig. 5) and sequences were decoded by Sanger's cycle sequencing method.

The sequence was then checked for similarities in NCBI database by BLASTn. The 16S rRNA gene sequence was also submitted to the NCBI database (Accession number: OR398534). The isolate was found to have the highest similarity (100%) with *Citrobacter freundii* strain GMU8049 (Fig. 6) hence considered as *Citrobacter freundii*.

4. Discussion:

Multiple antibiotic-resistant bacteria have caused severe worries about potential risks to the public's health due to their prevalence in food products, particularly eggs. This study focuses on the emerging threat of multi-antibiotic-resistant *Citrobacter freundii* in eggs, a Gram-negative bacteria with a history of developing antibiotic resistance. The research revealed a high level of multi-antibiotic-resistant *Citrobacter freundii* in eggs, suggesting possible food supply chain contamination. The isolation of these strains from eggs has raised concerns about the spread of these hard-to-treat pathogens to consumers.

Based on morphological and biochemical traits, 30 out of 50 egg samples (about 60%) tested positive for gram-negative bacteria. Eighty-four percent (84%) of the acquired isolates (30) were discovered in eggshells, while sixteen percent (16%) were discovered in egg yolk and egg white components. This is an alarming finding.

Among all isolates, multidrug-resistant strain *Citrobacter* spp. was identified and classified based on their 16S rRNA gene sequence (Fig. 6). The sequence similarities were examined by NCBI–BLAST. The 16S rRNA gene sequence was submitted to the NCBI database (Accession number: OR398534). The *Citrobacter* isolate was found to have the highest similarity (100%) with *Citrobacter freundii* strain GMU8049 (Fig. 6). There are many published reports of isolation of antibiotic-resistant *Citrobacter freundii* from eggs from Bangladesh (Fardows & Shamsuzzaman, 2015) as well as from worldwide (Jahantigh, 2013; Musgrove et al., 2008; Papadopoulou et al., 1997). Yet, a complete understanding of the prevalence and mechanisms of persistence of *Citrobacter freundii* in eggshell and egg content is lacking to a great extent.

The results of the antibiotic sensitivity test revealed that pathogens were highly resistant to Erythromycin (90%), Amoxicillin (77%), Cefixime (57), Chloramphenicol (50%), and Azithromycin (43%). However, they were sensitive to Imipenem (93%), Gentamycin (70%), and Ciprofloxacin (60%) and a lower sensitivity to Azithromycin (46%). Several previous reports have also implicated that eggs might be a reservoir of multi-antibiotic-resistant *Citrobacter* spp. (Fardows & Shamsuzzaman, 2015; Jahantigh, 2013; Papadopoulou et al., 1997). This finding also aligns with previous studies highlighting the role of food products as potential reservoirs and vehicles for the dissemination of antibiotic-resistant bacteria (Sultana et al., 2014). The multi-antibiotic resistance profile found in *Citrobacter freundii* isolates is particularly concerning since it undermines the efficacy of conventional treatment options by conferring resistance to several types of antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides (Johnson et al., 2020). To comprehend the genetic foundation and modes of transmission of these resistant strains, it is important to grasp better the fundamental mechanisms causing this resistance.

The silent nature of this public health threat is evident in the lack of routine surveillance and screening for multi-antibiotic-resistant *Citrobacter freundii* in food products. The study highlights the need for enhanced monitoring and regulatory measures to ensure the safety of the food supply chain and mitigate the potential risk of antibiotic-resistant infections in consumers (Rodriguez-Mozaz et al., 2015).

A One Health approach is crucial to tackle this issue comprehensively, involving collaboration between human health, animal health, and environmental sectors. Promoting responsible antibiotic use in both human and veterinary medicine is also crucial to prevent the further emergence and spread of multi-antibiotic-resistant strains (Collignon et al., 2019; Liu et al., 2017; Velazquez-Meza et al., 2022).

In summary, the research sheds light on the emerging threat of multi-antibiotic-resistant *Citrobacter freundii* in eggs, indicating a potential route for transmission to humans and posing challenges to effective treatment options. Timely and coordinated efforts from various stakeholders are necessary to mitigate this public health threat and safeguard the well-being of consumers and the broader community.

4. Conclusion:

Our study concludes that the existence of multi-antibiotic-resistant *Citrobacter freundii* in eggs poses a subtle but serious hazard to the public's health. The significance of this covert threat to the public's health necessitates a swift response from the scientific community and decision-makers. To track the frequency and spread of these infections, increased surveillance and monitoring of antibiotic-resistant bacteria in food products, particularly eggs, is crucial. Furthermore, it is crucial to put rigorous regulatory controls in place to guarantee food safety and stop the spread of resistant strains among consumers.

Author Contributions

JA, MAM, MF, and MAS were involved in the research concept and design; JA, AC, and EJ conducted the literature search and data extraction; JA, MF, and MAS took part in the collection and/or assembly of data; MF, MAS, JA, EJ, and MAM were involved in data analysis and interpretation; MF, JA, and MAS took part in writing the article; and MAM, EJ, and AC were involved in the critical revision of the article. All authors have read and approved the manuscript.

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Competing financial interests

The author has no conflict of interest.

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