PREVALENCE OF GRAM-POSITIVE BACTERIA IN HOSPITAL CAFETERIA FAST FOODS IN DHAKA, BANGLADESH AND THEIR RESISTANCE TO CURRENT ANTIBACTERIAL AGENTS

Bioresearch Communications
Volume 10, Issue 1, January 2024

DOI:
doi.org/10.3329/brc.v10i1.70644

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ABSTRACT

Emerging antibiotic resistance of Gram-positive bacteria leading to strikethrough infections are one of the major threats to global public health. This research aimed to determine different circulating Gram-positive bacterial species as well as their antibiotic resistance pattern along with their phylogenetic analysis. Fifty (50) Gram-positive isolates from different bacterial species were isolated from eight different hospital cafeteria fast foods of Dhaka, Bangladesh of which 58% of the isolates were from sandwiches and 42% of the isolates were from burger samples. The isolates were divided into six different groups based on different biochemical tests belonging to *Bacillus spp.* followed by *Planococcus spp.*, *Micrococus spp.*, *Streptococcus spp*., *Clostridium spp.* and *Staphylococcus spp.* Distribution of isolates varied greatly keeping the highest number of isolates from *Bacillus spp.* and lowest in *Staphylococcus spp.* BLAST search and phylogenetic analysis revealed that all sequenced isolates were Gram-positive bacteria under firmicutes and shared their identity with *Chryseomicrobium* spp., *Sporosarcina psychrophila*, *Bacillus licheniformis.* About 6% of the isolates were strong biofilm former whereas 4%, 24% and 66% of the isolates were moderate biofilm formers, weak biofilm formers and non-biofilm formers respectively. About 6% of the isolates showed β-hemolytic properties. About 72% of the isolates were multi-drug resistant. Among the antibiotics, ampicillin and penicillin showed the highest level of resistance followed by cephalexin, nitrofurantoin, meropenem and no resistance to imipenem. A higher proportion of multi-drug resistant (MDR) Gram-positive isolates were revealed from the hospital cafeteria fast foods demonstrating the risky situation regarding hospital associated infections caused by Gram-positive bacteria.

KEYWORDS: fast foods, hospital cafeteria, Gram-positive bacteria, antibiotic resistance Amino acid profile, essential amino acid content, recommended nutrient intake.

RECEIVED: 24 July 2023, ACCEPTED: 11 October 2023

TYPE: Original Research

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Introduction

Gram-positive organisms exhibit a wide range of growth and resistance patterns (Wisplinghoff et al., 2003). The capacity of the thick peptidoglycan (PG) layer to hold the dye allows for the identification of Gram-positive bacteria using crystal violet dye, which reacts with the bacteria to produce blue color when viewed under a microscope (Sizar et al., 2023). The cell wall of Gram-positive bacteria varies from that of Gram-negative bacteria in that it has a thick layer of PG that surrounds the plasma membrane to shield Gram-positive bacteria from the hostile environment in which they reside (Karaman et al., 2020) (Silhavy et al., 2010). The fatality rates from bloodstream infections have risen by 78% in just the last two decades (National Nosocomial Infections Surveillance System, 2004). Wide variety of diseases can result from Gram-positive infections (GPIs), including skin and soft tissue infections, surgical and trauma wound infections, urinary tract infections, gastrointestinal tract infections, pneumonia, osteomyelitis, endocarditis, thrombophlebitis, mastitis, meningitis, toxic shock syndrome, septicaemia, and infections of indwelling medical device (Kulkarni et al., 2019). In the modern, globalized society, food-borne diseases (FBDs) have recently become a significant public health concern, and their effects on both health and the economy (due to their high rates of illness and mortality) are increasingly recognized (Abebe et al., 2020). Bangladesh has experienced a rise in the consumption of fast food, similar to other Asian countries, as a result of globalization, economic growth, and rising incomes (Islam & Ullah, 2010). Fast food contamination with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be

transferred to other bacteria of human clinical significance. Treatment of bacterial infections with the currently available antibiotics has become extremely difficult due to the rise of multidrug-resistant bacteria (Karaman et al., 2020). In addition to these, hospitals use a lot of antibiotics, which means that bacteria from patients receiving treatment could contaminate the environment in the cafeteria with strains that are resistant to antibiotics (Ghosh & Alim, 2022). Different pathogenic Gram-positive bacteria from different bacterial species such as *Staphylococcus spp., Streptococcus spp., Enterococci spp., Clostridium spp., Bacillus spp., Corynebacterium spp., Listeria spp.* are particularly notable today for posing problems with resistance, significantly burdening the public health system, and increasing healthcare costs (Kulkarni et al., 2019), (Woodford & Livermore, 2009) . Therefore, this study aims to investigate the circulating diverse Gram-positive microorganisms and their antibiotic resistance pattern in fast foods from hospital cafeteria of Dhaka, Bangladesh.

Materials and Methods

Location of the study:

Eight different hospital cafeterias (4 governmental and 4 non-governmental) of Dhaka city, Bangladesh were studied in this study. The hospital cafeterias were located in A (23°42'40.1"N, 90°24'06.6"E), B (23°43'44.7"N, 90°24'11.7"E), C (23°46'25.8"N 90°22'45.5"E), D (23°46'26.8"N 90°22'23.4"E), E (23°44'24.6"N 90°23'48.6"E), F (23°44'18.5"N 90°23'49.4"E), G (23°44'22.8"N 90°24'34.0"E) and H (23°45'02.1"N 90°22'12.6"E) sites. As large number of population of different socioeconomic status admit into those hospitals along with their attendant, relatives, hospital stuffs; these hospital cafeterias were chosen.

Sample collection

Total 80 samples, 5 samples of sandwich and 5 samples of burger from each of 8 different hospital cafeterias were selected for the study. Sandwiches and burgers were selected because of their increasing popularity and moist and raw ingredients that may harbor different pathogenic organisms.

Study period

All the samples of the study were collected between August 2019 and February 2021.

Processing and preparation of samples

The samples were collected, wrapped, and chilled in an icebox (at 4 ^{0}C) before being processed in the lab within two hours. Sandwich and Burger were removed from their sterilized plastic bags, placed in sterile petri dishes, and weighed to a weight of 10 grams using a balance. 10 grams of each sample were combined with 90 mL of peptone water that had already been prepared (Clarence et al., 2009), plugged with cotton, and shaken to homogenize the mixture. After thoroughly mixing the sample, 1 mL of the sample solution was pipetted into test tubes with cotton plugs that had been sterilized and contained 9 mL of peptone water with a 0.1% concentration. They were thoroughly combined after that by shaking. So, after preparing the initial homogenate dilution, additional serial dilutions up to the fifth dilution were made from this homogenization.

Isolation and storage of bacterial isolates

50 µl of the homogenate of the samples of each dilution was used to plate samples on various agars using the spread plate technique, including Plate Count Agar (PCA), Nutrient Agar (NA), Mannitol Salt Agar (MSA), Phenylethyl Alcohol Agar (PEA), Citrate Agar, Blood Agar and cooked meat media. (Himedia, India). Following that, normal plates with 25–250 colony counts were performed after 24 hours of incubation at 37 °C. Colony forming units per milliliter (cfu/ml) were used to express the counts for each plate. To obtain pure cultures of the isolates, discrete colonies were aseptically subcultured into differential and selective media. The resulting growth's pure isolates were then kept at 4°C (Jahan et al., 2016).

Identification of bacterial isolates

In accordance with the guidelines in Bergey's Manual of Determinate Bacteriology ("Bergey's Manual of Determinative Bacteriology.," 1924) , selected pure isolates were then subjected to Gram's staining, followed by cultural, morphological, and biochemical tests like Kligler Iron Agar (KIA), Motility Indole Urease (MIU), Nitrate reduction, Citrate use, Oxidase, Catalase, coagulase, and Methyl Red-voges-Proskauer for their presumptive identification (Levine, 1975).

Antibiotic sensitivity pattern of the isolates

Using 17 different antibiotics from 11 different antibiotic classes, the antibiotic sensitivity of all the isolates was analyzed. The antibiotics included in the study were penicillins (ampicillin, 25 µg; penicillin, 10 µg); aminoglycosides (gentamycin, 10 µg; kanamycin, 30 µg; streptomycin, 10 µg); cephalosporins (ceftriaxone, 30 µg; cephalexin, 30 µg); chloramphenicol (chloramphenicol, 30 µg); macrolides (azithromycin, 30 µg); polypeptides (colistin, 10 µg); quinolones (ciprofloxacin, 5 µg; levofloxacin, 5 µg); sulfonamides (sulphamethoxytrimethoprim, 25 µg); tetracycline (tetracycline, 30 µg); nitrofuran (nitrofurantoin, 300 µg) , carbapenem (imipenem, 10 µg ; meropenem, 10 µg) (Himedia, India; Titan media, India). These doses of different antibiotics were used because in these doses the antibiotics were expected to be clinicallyb effective against different bacterial infections that is these doses increases the therapeutic effectiveness of different antibiotics (Kowalska-Krochmal & Dudek-Wicher, 2021; (*Clinical & Laboratory Standards Institute*,2022) The isolates' susceptibility to various types of antibiotics was tested using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar(Abbey & Deak, 2019). Although the disc diffusion method for colistin has some drawbacks because the colistin powder was not readily available, it had been used, according to Dey et al., 2018 (Dey et al., 2018). According to Clinical and Laboratory Standards Institute break points, the results were finally classified as susceptible, intermediate, and resistant(*Clinical & Laboratory Standards Institute*, 2022). Multidrug-resistant (MDR) strains are those that are resistant to five or more antibiotics (Hoque et al., 2018).

Phenotypic virulence assays

Biofilm formation assay

Standard methods were used to assess the biofilm formation potential of the isolates using 96-well microtiter plate assays. The study measured the absorbance of solubilized crystal

violet (CV) on a plate reader at 600 nm using 30% acetic acid in water as the blank and TSB as the control. In the 96-well tissue culture plates, the assay was run twice. To evaluate the BF capacity of isolates on a 4-grade scale, the cut-off optical density (ODc), which was established as three standard deviations above the mean optical density of the negative control, was used. According to the scale, non-biofilm formers, NBF (OD ≤ ODc); weak biofilm formers, WBF (ODc < OD ≤ 2 × ODc); moderate biofilm formers, MBF (2 × ODc < OD ≤ 4 × ODc), and strong biofilm formers, SBF (OD > 4 × ODc) (Hoque et al., 2020).

Hemolysin assay

The hemolytic activity of the isolates was tested by streaking blood agar base plates with 5% sheep blood addition. After 24 hours at 37 degrees Celsius, the plates were examined for signs of a hemolysis pattern: β-hemolysis (clear zones around colonies), α-hemolysis (a green zone around colonies), or γ-hemolysis (no halo around colonies) (Saha et al., 2020).

Molecular identification of the isolates

Chromosomal DNA of the isolates was extracted by boiling DNA method (Saha et al., 2020) followed by amplification of the 16S rRNA gene of the isolates by polymerase chain reaction (PCR). Selected isolates were sequenced followed by phylogenetic analysis to find out their close relatives.

Extraction of chromosomal DNA

On nutrient agar plates, isolates were revived, and one colony of each isolate was cultured on nutrient broth. The isolate's genomic DNA was extracted using the boiled DNA extraction technique from overnight cultures (Saha et al., 2020). The samples were briefly centrifuged at 15,000 g for 15 min, the supernatant was thrown away, and the process was repeated. The pellets were once more suspended in 200 l of filtered (22 microliter), distilled water, the supernatant was once more removed, and they were then heated to 100 °C in a heat block for 10 minutes, cooled on ice for 10 minutes, and centrifuged at 15,000 g for 10 minutes. Finally, a fresh, sterile eppendrof tube was used to collect 120–150 microliters of supernatant. The amount and purity of the isolated DNA (1.8±0.3) were calculated using 260/280 absorbance ratios.

Polymerase Chain Reaction (PCR) of 16SrRNA gene

The 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR) using universal primers (27F,5'-AGAGTTTGATCMTGGCTCAG-3'and1492R,5'-TACGGYTACCTTGTTACGACTT-3′) from extracted chromosomal DNA of the isolates (Weisburg et al., 1991). The successful amplicons were visualized using the Image ChemiDocTM Imaging System (Bio-Rad, Hercules, CA, USA) after being electrophoresed on 1% agarose (Sigma, USA) gel at 100 volts, 200 mA for 30 minutes (Hoque et al., 2019).

Sequencing and Phylogenetic analysis

The successful 16S rRNA gene amplicons of selected isolates were sequenced by Macrogen Inc. Seoul, South korea.

Alignment of sequences and Identification

The SeqMan Genome Assembler (DNAstar, USA) was used to combine partial sequences of the 16S rRNA gene from the chosen isolates that were obtained using the forward and reverse primers (27F and 1492R). Using the basic local alignment search tool (BLAST), the 16S rRNA sequence of the isolates was compared with the GenBank database of the National Center for Biotechnology Information (NCBI).

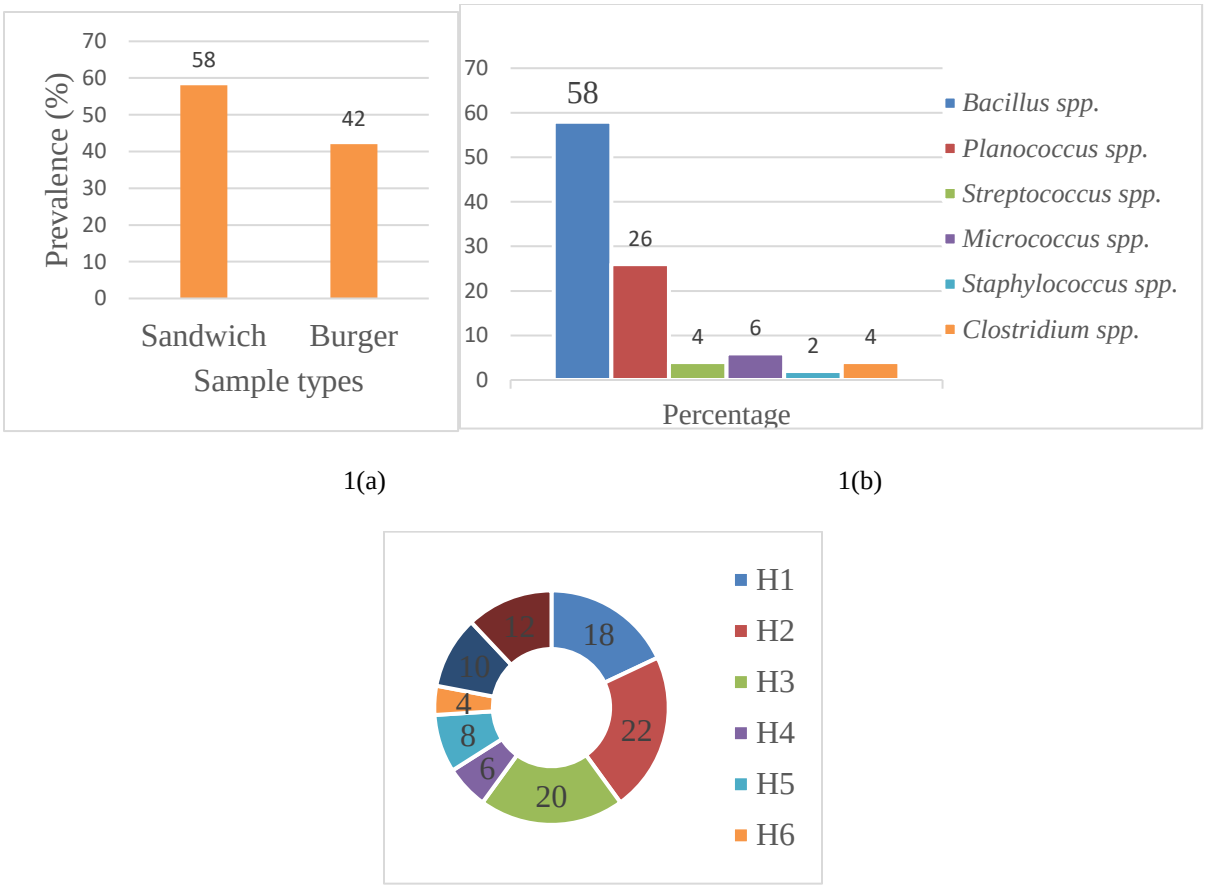
Consturction of Phylogenetic tree

Using MEGA11 (Tamura et al., 2021), phylogenetic analysis of the isolates' 16S rRNA sequences was carried out by retrieving reference sequences from the NCBI database at http://www.ncbi.nlm.nih.gov. Using ClustalW, the sequences were aligned and cleaned up. The Neighbor joining algorithm was used to construct a phylogenetic tree from the aligned sequences (Saitou & Nei, 1987) and Kimura-2 parameter model with 1000 bootstrap replications (Kimura, 1980) . The branch was accompanied by the proportion of replicate trees in which the associated taxa clustered during the bootstrap test (1000 replicates).

Results

Distribution of the isolates

50 Gram-positive isolates of different bacterial species were isolated from the burger and sandwich samples of eight different hospital cafeterias Dhaka, Bangladesh. About 58% (29) of the isolates were from sandwich and 42% (21) of the isolates from burger (Figure 1a). The isolates distributed widely among different bacterial species keeping the highest number of isolates in *Bacillus spp.* (58%) followed by *Planococcus spp.* (26%), *Micrococus spp.* (6%), *Streptococcus spp*. (4%), *Clostridium spp.* (4%) and *Staphylococcus spp.* (2%) (Figure 1b). The isolates distributed greatly among different hospital cafeterias keeping the highest number of isolates in hospital 2 that is H2 (22%) followed by H3 (20%), H1 (17%), H8 (12%), H7 (10%), H5 (8%), H4 (6%) and lowest number in H6 (4%) (Figure 1c).



1(a) 1(b)

1(c) Here, H for Hospital cafeteria.

Figure 1. Prevalence of Gram-positive isolates in hospital cafeteria fast foods in Dhaka, Bangladesh. a) Bar plot showing the prevalence of isolates in different types of fast food samples b) column chart showing the distribution of isolates among different bacterial species and c) pie chart showing the prevalence of isolates in different hospitals.

Phenotypic characterization of the isolates

Phenotypic characterization were done by Gram staining method followed by different biochemical tests such as Kligler Iron Agar (KIA), Motility Indole Urease (MIU), nitrate reduction, Methyl Red-Voges-Proskauer (MR-VP), citrate use, catalase, coagulase and oxidase tests and the results of these tests were presented in Table 1.

Table 1. Biochemical characteristics of Gram positive bacteria (representative isolate of each group) isolated from burger and sandwich samples of selected hospital cafeterias

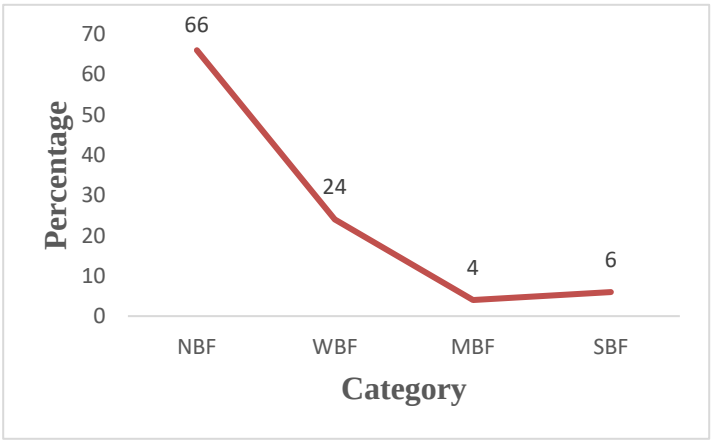
ID of the isolates	Gram reaction	Agar slant culture characteristics	KIA				MIU			Nitrate reduction	MR-VP		Citrate use	Catalase	Oxidase	Coagulase	Presumed organism
			Slant	Butt	Gas	H_2S	Motility	Indole	Urea		MR	VP					
H8-131S (G-1)	Rod (Long) (+)	Thin, even, grayish growth	-	+	-	-	+	-	-	+	-	-	-	+	-	*N/D*	*Bacillus* spp.

H3-65B (G-2)	Coccus (+)	Slimy, white, somewhat translucent growth	-	+	-	-	-	-	+	+	-	-	+	+	-	*N/D*	*Planococcus* spp.
H6-99S (G-3)	Coccus (+)	Abundant, white, thick, glistening growth	+	+	-	-	+	-	-	-	+	+	-	-	-	*N/D*	*Streptococcus* spp.
H2-28S (G-4)	Coccus (+)	Thin, even, grayish growth	-	+	-	-	-	-	+	+	+	-	-	+	+	*N/D*	*Micrococcus* spp.
H4-84B (G-5)	Coccus (+)	Abundant, white, thick, glistening growth	+	+	-	-	-	-	-	+	+	-	-	+	-	-	*Staphylococcus* spp.
H1-19B (G-6)	Rod (+)	Smooth, low convex, moist, translucent, gray with a shiny surface.	-	+/-	+/-	+	+	-	-	-	-	-	-	+	-	*N/D*	*Ciostridium* spp.

* Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, B & S= Burger and Sandwich respectively, the number represents the number of the isolates.
*Details of the groups and Id & biochemical characteristics of the isolates are in Suppl. Table 1and Suppl. Table 2.

Biofilm formation capacity of the isolates

In this study, the average optical density (OD) of the negative control was 0.209 ± 0.043 and the cut off OD value was set as 0.34. By comparing the category of biofilm former (BF), 66%, 24%, 4%, 6% isolates were non-biofilm formers (NBF), weak biofilm formers (WBF), moderate biofilm formers (MBF) and strong biofilm formers (SBF) respectively



Here,

NBF= Non-biofilm formers

WBF= Weak biofilm formers

MBF= Moderate biofilm formers and

SBF= Strong biofilm formers.

Figure 2. Biofilm formation capacity of the isolates

Hemolytic properties of the isolates

Blood agar base plates with 5% sheep blood was used to observe the hemolytic properties of the isolates. About 6% of the isolates showed complete hemolysis (β-hemolysis) that is clear zones around colonies and 10% of the isolates showed partial hemolysis (α-hemolysis) that is a green-hued zone around the colonies. Majority (84%) of the isolates showed no hemolysis (γ-hemolysis) that is no halo around colonies.

Table 2. Hemolytic properties of the isolates

α- hemolysis	β- hemolysis	γ- hemolysis
H2-33B, H3-53S, H6-102S, H7-118B, H8-129S	H3-46S, H3-65B, H8-131S	H1-2S, H1-4S, H1-9S, H1-10S, H1-11S, H1-12B, H1-15B, H1-17B, H1-19B, H2-23S, H2-26S, H2-27S, H2-28S, H2-31B, H2-34B, H2-35B, H2-37B, H2-38B, H2-40B, H3-42S, H3-43S, H3-45S, H3-51S, H3-54S, H3-55S, H3-56S, H4-71S, H4-72S, H4-84B, H5-90B, H5-91B, H5-92B, H5-93B, H6-99S, H7-111S, H7-112S, H7-116B, H7-117B, H8-134B, H8-135S, H8-136S, H8-137S.

Antibiogram susceptibility pattern of the isolates

17 antibiotics from 11 different antibiotic classes were used for investigation of antibiotics susceptibility of the isolates. The study isolates displayed a comparatively larger percentage of resistance against specific antibiotic classes, according to the antimicrobial resistance profile, including ampicillin (100%), penicillin (100%) followed by cephalexin (86%), then nitrofurantoin and meropenem (84% each), azithromycin (52%), colistin (46%), ceftriaxone (24%), tetracycline (20%), sulphamethytrimethoprim (10%), ciprofloxacin (10%), levofloxacin (10%), kanamycin (6%), chloramphenicol (8%), , streptomycin (6%), gentamycin (6%) and highly sensitive to imipenem

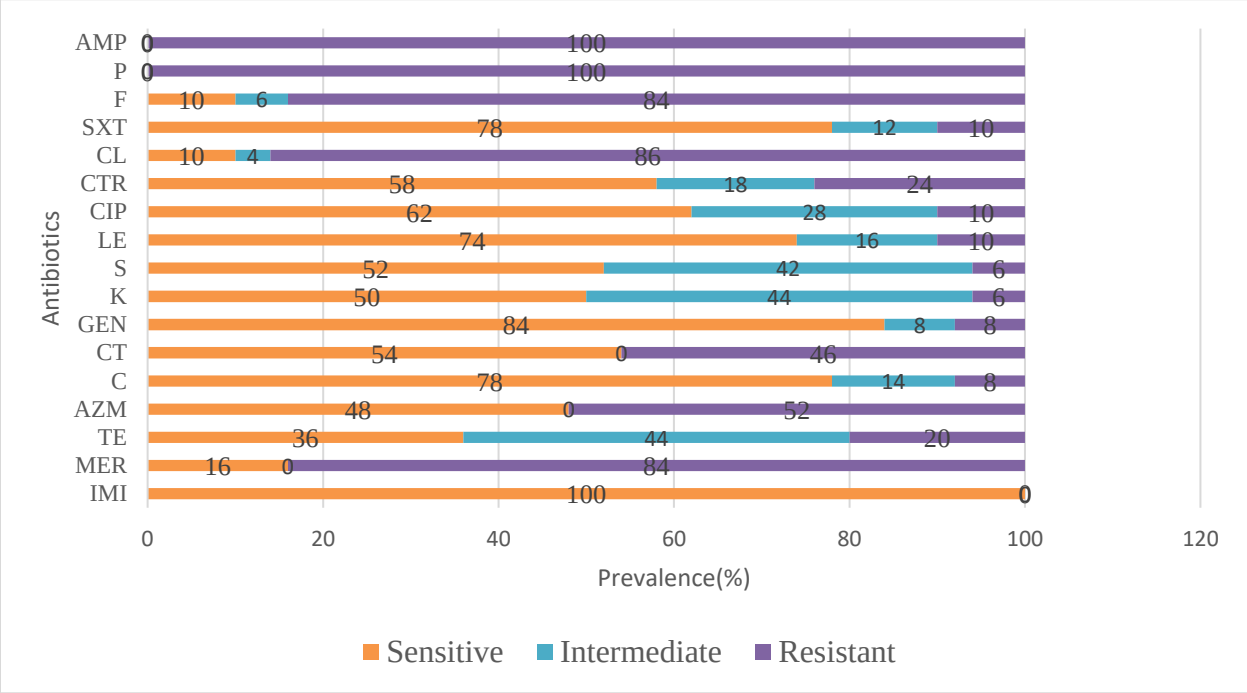


Figure 3. Antibiotic susceptibility pattern of the isolates. MI-Imipenem; MER-Meropenem; TE-Tetracycline; AZM-Azithromycin; C-Chloramphenicol; CT-Colistin; GEN-Gentamycin; K-Kanamycin; S-Streptomycin; LE-Levofloxacin; CIP-Ciprofloxacin; CTR-Ceftriaxone; CL-Cephalexin; SXT-Sulphamethoxytrimethoprim; F-Nitrofurantoin; P-Penicillin and AMP-Ampicillin. Categorization of sensitive, intermediate and resistant is based on CLSI guideline (*Clinical & Laboratory Standards Institute*, 2022.).

Among the isolates about 28% of the isolates were resistant to less than 5 antibiotics, 66% of the isolates were resistant to 5-11 antibiotics and 6% of the isolates were resistant to twelve or more antibiotics.

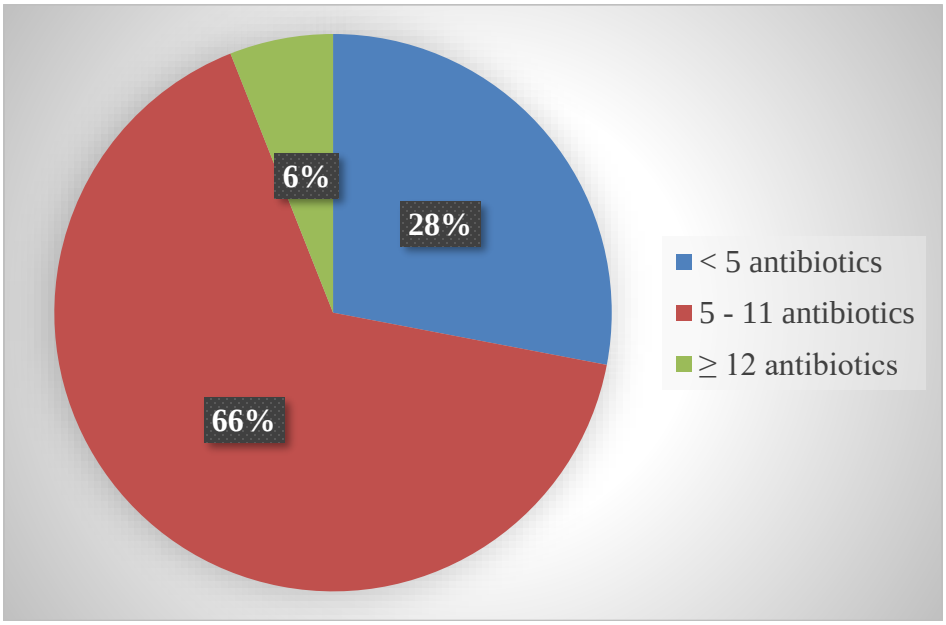


Figure 4. Prevalence of resistant isolates among different antibiotics.

Phylogenetic distribution of the isolates

16S rRNA gene phylogeny of the isolates

Based on the abundance of the isolates, their antibiotic resistance pattern, phenotypic virulence assays that is biofilm formation capacity, hemolytic pattern; three isolates (from most abundant groups, resistant to 12 or more antibiotics, strong biofilm former and β-hemolysis) were selected as representatives to analyze their phylogenetic correlation to the nearest species level by detailed 16S rRNA gene sequence analysis. The isolates selected were H3-46S (G-1), H3-65B (G-2) and H8-131S (G-1).

The 16S rRNA amplicons were sequenced and identified using BLAST analysis. The accession number of NCBI submitted sequences are from OR486967 to OR486969 and the BLAST results were in **Table 3.**

BLAST search and phylogenetic analysis revealed that all of three isolates were Gram-positive bacteria under firmicutes. Isolate H3-46S of G-1 was phylogenetically closely related to *Chryseomicrobium* spp. with 99.62% 16S rRNA gene sequence identity. H3-65B of G-2 shared 99.85% identity to *Sporosarcina psychrophila* and H8-131S of G-1 were closely related to *Bacillus licheniformis* with 99.93% identity (Figure-6).

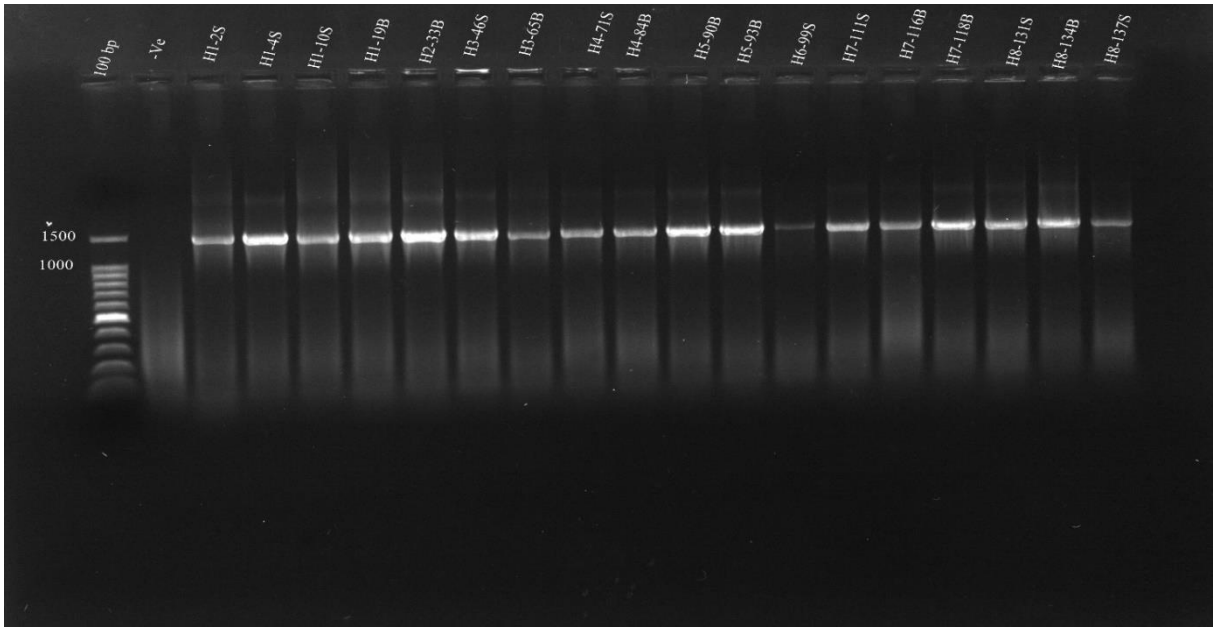


Figure 5. Representative figure of 16S rRNA PCR. Lane 1 is 100 bp ladder (Promega, USA), lane 2 is negative control, lane 3-20 is representative Gram-positive isolates (Product size-1465 bp).

Table 3. Maximum identity profile of 16s rRNA gene sequences of selected isolates according to BLAST identification.

H3-46S (G-1)	*Chryseomicrobium* sp. strain YL2 (KY855388.1)	99.62%	Firmicutes
H3-65B (G-2)	*Sporosarcina psychrophila* strain DSM 6497 (KU569324.1)	99.85%	Firmicutes
H8-131S (G-1)	*Bacillus licheniformis* strain YS65 (KU551256.1)	99.93%	Firmicutes

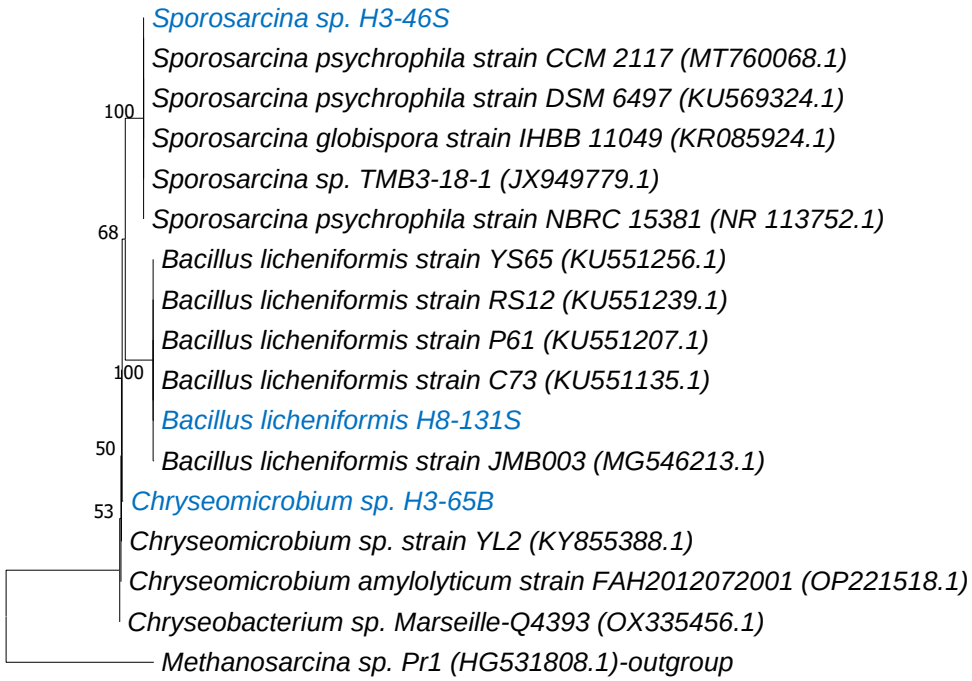


Figure 5: Phylogenetic tree of 16S rRNA gene sequences of gram-positive isolates from hospital cafeteria fast foods and close relative reference isolates retrieved from database with accession numbers. The tree was generated in program MEGA11 using the Neighbour-Joining algorithm with the *Methanosarcina sp.* sequence as outgroup. Bootstrap values (n =1000 replicates) are shown at branch nodes. Isolates in this study are indicated with symbol in the beginning. Here, H1, H2, H3, H4, H5, H6, H7, H8= Name of the hospital, B & S= Burger and Sandwich respectively, the number represents the number of the isolates.

Discussion

Gram-positive infections (GPIs) caused by emerging antimicrobial resistance are one of the biggest global threats to public health. Multidrug resistant bacteria can cause GPIs, which can lead to higher rates of morbidity and mortality as well as more expensive and longer hospital stays (Kulkarni et al., 2019). Gram-positive bacteria are responsible for causing significant infections in hospitals (Kulkarni et al., 2019). Wide range of illnesses, including infections of the skin and soft tissues, surgical and trauma wounds, urinary and gastrointestinal tracts, pneumonia, osteomyelitis, endocarditis, thrombophlebitis, mastitis, meningitis, toxic shock syndrome, septicaemia, and infections of indwelling medical devices are caused by Gram-positive bacteria (Kulkarni et al., 2019).

Abundance of different Gram-positive bacterial species is increasing day by day. In this study, the most prevalent bacterial species was *Bacillus spp*. followed by *Planococcus spp*. Different *Bacillus spp.* have been linked to human foodborne illness. Information about the virulence mechanisms of *Bacillus spp.* is scanty and their risk is underestimated (Fangio et al., 2010). Although the prevalence of Staphylococcus and Streptococcus spp. are most prevalent in food borne diseases among Gram-positive bacteria (Freitas et al., 2023; Katzenell et al., 2001; Kadariya et al., 2014); the presences of these species in the present study was very low. The coagulase positive staphylococcus species that is *Staphylococcus aureus* is the most common human pathogenic organism among Gram-positive bacteria that cause infectious diseases (Guo et al., 2020). But the Staphylococcus spp. found in this study was coagulase negative. Coagulase-negative staphylococci (CoNS) are part of normal human skin flora. Generally they are considered as harmless when it remains outside the body. However, the bacteria can cause infections when present in large amounts, or when present in the bloodstream (Michels et al., 2021) .The pathogenic streptococcus species possess that β-hemolytic properties, generally they are pathogenic. But the Streptococcus spp. found in this study were possess no hemolytic activity.

The antibiogram resistance pattern of the isolates showed that most of the isolates were resistant to multiple antibiotics. The strain that is resistance to five or more antibiotics is defined as Multidrug-resistance (MDR) (Hoque et al., 2018). About 72% of the isolates were Multidrug-resistance (MDR) (Figure-4). The study isolates displayed a comparatively larger percentage of resistance against specific antibiotic classes, according to the antimicrobial resistance profile, including complete resistance in ampicillin and penicillin followed by cephalexin, then nitrofurantoin and meropenem, azithromycin, colistin and highly sensitive to imipenem.. A growing global crisis is pathogen resistance to the available antibiotics. Some of the most common resistant pathogens that present serious clinical challenges are gram-positive bacteria. Gram-positive MDR bacteria are significant human pathogens that are responsible for both community- and healthcare-associated infections (Karaman et al., 2020). About 6% of the isolates were strong biofilm former and possessed β-hemolytic properties. 4%, 24% and 66% of the isolates were moderate biofilm formers, weak biofilm formers and non-biofilm formers respectively. 84% and 10% of the isolates showed γ-hemolysis and α-hemolysis respectively.

Based on the abundance of the isolates, their antibiotic resistance pattern, phenotypic virulence assays that is biofilm formation capacity, hemolytic pattern; three isolates were selected as representatives to analyze their phylogenetic correlation to the nearest species level. The most prevalent bacterial species was *Bacillus spp*. followed by *Planococcus spp*.H3-46S (G-1), H8-131S (G-1) isolates were from *Bacillus spp*. And H3-65B (G-2) was from *Planococcus spp.*. These three isolates were resistant to 12 or more antibiotics that is highly antibiotic resistant isolates. These three isolates were strong biofilm former and possessed β-hemolytic properties. The development of biofilms is a crucial component of pathogenicity and increases resistance to several kinds of antimicrobials(Hoque et al., 2020). β-hemolysis is one of the most important virulence agents than can cause infections to the human (Humaidan, 2021). Among all the isolates, because of the possibility of these three isolates to be pathogenic based on the phenotypic characteristics, were selected for phylogenetic analysis. BLAST search and phylogenetic analysis revealed that all of three isolates were Gram-positive bacteria under firmicutes. Isolate H3-46S of G-1 was phylogenetically closely related to *Chryseomicrobium* spp. with 99.62% 16S rRNA gene sequence identity. H3-65B of G-2 shared 99.85% identity to *Sporosarcina psychrophila* and H8-131S of G-1 were closely related to *Bacillus licheniformis* with 99.93% identity (Figure-6) (Table-3).

Conclusion

The study reveals prevalence of different Gram-positive bacterial species along with their antibiotic resistance profile and their phylogenetic distribution in hospital cafeteria fast foods in Dhaka, Bangladesh. Diverse Gram-positive bacterial species along with higher percentage of multi-drug resistant bacteria was present in hospital cafeteria fast foods. However, further research should be done with more hospital cafeterias and with different region for better understanding of the situation.

Author Contributions

Conceptualization, S.G., M.S and S.R.A.; formal analysis, S.G and H.A.; writing – original draft preparation, S.G.; Writing-review and editing, S.G., H.A, M.S and S.R.A.; supervision, M.S, M.A.H. and S.R.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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