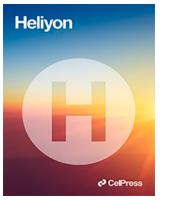
Heliyon 9 (2023) e21584

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Dynamics of antimicrobial resistance and virulence of staphylococcal species isolated from foods traded in the Cape Coast metropolitan and Elmina municipality of Ghana

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ARTICLE INFO

Keywords:
Polymerase chain reaction
Whole-genome shotgun sequencing
Antimicrobial susceptibility
Virulence
Multiple drug resistance
Staphylococcus species

ABSTRACT

The impact of staphylococci on food poisoning and infections could be higher than previously reported. In this study, we characterised the occurrence and coexistence of antimicrobial resistance and virulence genes of staphylococci isolates in foods. Staphylococci were isolated from 236 samples of selected street-vended foods and identified. The pattern of antimicrobial resistance and virulence genes in the staphylococci were assessed using disc diffusion, PCR and analysis of next-generation sequencing data. The food samples (70.76 %) showed a high prevalence of staphylococci and differed among the food categories. Forty-five *Staphylococcus* species were identified and comprised coagulase-negative and positive species. *Staphylococcus sciuri* (now *Mammaliicoccus sciuri*), *S. aureus*, *S. kloosii*, *S. xylosus*, *S. saprophyticus*, *S. haemolyticus* and *S. succinus* were the most abundant species. The staphylococcal isolates exhibited resistance to tetracycline, levofloxacin, ciprofloxacin, norfloxacin, gentamicin and amikacin and susceptibility to nitrofurantoin. Antimicrobial susceptibilities were also reported for cefoperazone, ceftriaxone, cefotaxime, nalidixic acid and piperacillin-tazobactam. The antimicrobial resistance and virulence genes commonly detected consisted of *tet*, *arl*, *macB*, *van*, *gyr*, *nor*, *optrA*, *bcrA*, *blaZ*, *taeA* and *S. aureus lmrS*. The isolates frequently exhibited multiple resistance (30.42 %) of up to eight antimicrobial drug classes. The isolates predominantly harboured genes that express efflux pump proteins (50.53 %) for antibiotic resistance compared with inactivation (10.05 %), target alteration (26.72 %), protection (7.67 %) and replacement (3.17 %). The virulence determinants comprised genes of pyrogenic toxin superantigens (*eta*, *etb*, *tst*), adhesions (*clf*, *fnbA*, *fnbB*, *cna*, *map*, *ebp*, *spA*, *vWbp*, *coa*) and genes that express exoproteins (nuclease, metalloprotease, γ-hemolysin, hyaluronate lyase). There was a statistically significant difference in the prevalence of staphylococci isolates and their antimicrobial resistance and virulence profile as revealed by the phenotypic, PCR and next-generation sequencing techniques. The findings suggest a higher health risk for consumers. We recommend a critical need for awareness and antimicrobial susceptibility and anti-virulence strategies to ensure food safety and counteract the spread of this clinically relevant genus.

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https://doi.org/10.1016/j.heliyon.2023.e21584
Received 3 April 2023; Received in revised form 20 October 2023; Accepted 24 October 2023
Available online 3 November 2023

1. Introduction

Street vended foods (SVFs) are crucial in the daily activities of many individuals and urban dwellers in developed and developing countries [1]. Though these foods are easily accessible, foodborne diseases and infections of microbial origins are commonly associated with them. SVFs are mainly produced by locals with less or inappropriate conditions for personal hygiene and temperature-holding capacities. Some vendors exhibit bad handling practices and limitations in their ability to protect foods from flies and dust from roadsides or bus terminals [1,2]. Reports on the prevalence of some foodborne pathogens such as *Salmonella* species, *Escherichia coli* and *Staphylococcus* species have been implicated in ready-to-eat foods [3,4].

Staphylococcus species have long occurred as natural residents on the skin and mucosae of animals and humans in a once harmless relationship with beneficial effects, including the expansion of the memory of T-cells, but now they are mostly implicated in life-threatening infections [5–7]. The pathogen has developed a complex regulatory network to control the evasion of antibiotics and ensure survival and adaptation to different environments [8]. The plasmids and transposons including the staphylococcal cassette chromosome *mec* (SCC*mec*) of the *Staphylococcus* genome encode antibiotic resistance genes while its phage-related and pathogenicity islands encode toxins and other virulence determinants [9,10]. Many of the antibiotic resistance determinants in *Staphylococcus* species occur by horizontal transmission between species from different environments and hosts [11,12].

Coagulase-positive staphylococci (CoPS) such as *Staphylococcus aureus* strains have been reported by several researchers to often exhibit high pathogenicity, produce superantigenic enterotoxins that cause food poisoning and display multiple drug resistance [10, 13,14]. Even though coagulase-negative staphylococci (CoNS) are widespread in food and have enterotoxigenic ability, their implication in the aetiology of foodborne illnesses has gained little attention [4,15]. The group has recently shown increased pathogenicity and antibiotic resistance and could be associated with a variety of infections when under-exposed primarily to infection-facilitating factors such as indwelling medical devices, skin trauma and immunocompromised hosts [16–18]. The growing inappropriate and indiscriminate use of antibiotics has brought about multidrug resistance (MDR), extensively drug resistance (XDR) and pan-drug resistance (PDR) in bacterial pathogens [19]. Currently, the increasing rate of spread of antimicrobial-resistant *Staphylococcus* species in foods threatens the ability to effectively treat staphylococcal infections and remains a global health and socio-economic concern [6,20].

The identification of staphylococcal species by phenotypic methods such as laboratory testing and the use of commercial kits, automated systems (including BD Phoenix and Vitek 2) and the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is quite laborious, limited to only some species and infrequently reliable because of the variability in the expression of some phenotypic characteristics by the species [15,21]. In this regard, molecular approaches such as PCR primer identification and next-generation sequencing (NGS) have been successfully and reliably employed to identify staphylococcal species [21,22]. The development of bioinformatics tools has similarly ensured the efficient characterization of target genes as well as their mechanisms of action [7,23]. This study was designed to narrow the knowledge gap on the diversity of *Staphylococcus* species recovered from SVFs in Ghana and their corresponding determinants of virulence and resistance to antimicrobial agents of therapeutic relevance. The study further assessed the potential associations between the observed phenotypes and genotypes relative to the parameters studied.

2. Materials and methods

2.1. Sample collection and staphylococcal isolation

A total of 236 most popular and easily accessible cooked foods (n = 148), fruits (n = 49) and vegetables (n = 39) were randomly collected from street food joints, and fruits and vegetable markets in the Cape Coast metropolitan and Elmina municipality of Ghana. Each food sample was collected three times at the same vendor from January to April 2021. The initial culturing and identification of staphylococci was done based on the methods previously described [24,25]. The cultures were serially diluted in buffered peptone water up to the 10^3 factor, cultured in Mannitol Salt Agar (MSA) medium (Oxoid, UK) using the pour plate technique and incubated at 37 °C for 48 h. All presumptive staphylococci colonies (yellow or red coloured colonies of 2–5 mm size) were subcultured and further analysed for microscopic characteristics, gram staining reaction, catalase production and coagulase activity. A single colony of each presumptive staphylococci isolate was subcultured in a nutrient broth medium (Oxoid, UK) and preserved in 10 % glycerol at 4 °C for further use.

2.2. Staphylococci isolates genomic DNA extraction

A 24 h culture of staphylococcal isolates was prepared in a nutrient broth medium and centrifuged for genomic DNA extraction according to the procedure described by Agyirifo et al. [26]. The extracted DNA was resolved in ethidium bromide-stained 1 % agarose gel electrophoresis at 90 V for 45 min and visualised in a GelDoc Go Imaging System (BIO-RAD, USA) to assess their quality. The concentration and purity of each DNA were determined in the 7415 nano spectrophotometer (Jenway, UK). The genomic DNA was then used for PCR analyses and next-generation sequencing (NGS) at the Beijing Genomic Institute (BGI), Hong Kong. The genomic DNA from all isolates was pooled and a replicate was obtained. The pooled genomic DNA and its replicate were sequenced using the whole-genome shotgun (WGS) sequencing method to obtain the NGS data.

The two cleaned sequence reads were submitted to the MG-RAST public database. The sequences were assigned the IDs 4963321.3 and 4963322.3. These sequence reads were used for taxonomy, antibiotic resistance and virulence factors profiling.

2.3. Identification of Staphylococcus species based on PCR

The identities of the isolates as staphylococci were confirmed at the genus level and the presence of *S. aureus* and CoNS in each food type was identified in a monoplex PCR. The primer sequences, annealing temperatures, and reaction volume and conditions used are shown in Table S1. The reaction mix comprised 10 μl 2× *Accurate Taq* master mix (Accurate Biotechnology Co. Ltd., China), 2 μl genomic DNA, 2 μl primer (reverse plus forward) and 6 μl PCR-grade water in a 0.2 ml tube. The PCR was done in a thermocycler (BIORAD, Singapore) and the products were resolved in ethidium bromide-stained 2 % agarose gel at 80 V for 40 min and visualised in a GelDoc Go Imaging System (BIORAD, USA). The reference strains used in this study are shown in Table S2. PCR-grade water was used as a negative control. The amplicons were scored against a 50 bp DNA ladder (Takara Bio Inc., China).

2.4. Antimicrobial susceptibility testing of staphylococci isolates

The susceptibility of the staphylococcal isolates to antibiotics was tested on Mueller-Hinton Agar (MHA) medium (HiMedia, India) using the Kirby-Bauer disk diffusion method and the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [27]. The bacterial cells were standardised in sterile physiological Saline at 0.5 McFarland and Axiom multidisc (Axiom Laboratories, India) consisting of 12 antibiotics used as therapy for staphylococcal infections were used for the test. The antibiotics comprised amikacin (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), gentamicin (10 μg), levofloxacin (5 μg), norfloxacin (10 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), piperacillin-tazobactam (75/10 μg) and cefoperazone (30 μg). The MHA plate cultures containing the antibiotic discs were allowed to stand for 30 min and incubated at 37 °C for 24 h. *Staphylococcus aureus* ATCC 29213 strain was used for quality control. Double distilled water (ddH_20) was used as a negative control. The testing was repeated three times and the zones of inhibition were measured. The isolates were categorised as susceptible or resistant to seven antibiotics according to the interpretative criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [28]. Currently, there is no susceptibility breakpoint applicable to staphylococcal species for cefoperazone, ceftriaxone, cefotaxime, nalidixic acid and piperacillin-tazobactam.

2.5. Detection of antibiotic resistance and virulence determinants based on PCR

The staphylococcal isolates were screened for antibiotic resistance and virulence genes using PCR. The genes considered for antibiotic resistance were *acrA*, *aac(3)-I*, *tetA*, *tetB*, *ermA*, *ermB*, *strA*, *vanA*, *mecA* and bla_{TEM}. Even though bla_{TEM} (extended-spectrum beta-lactamase) is normally produced by Gram-negative bacteria, recent studies have detected the enzyme in *Staphylococcus* species [29,30]. The virulence comprised genes for adherence factors (*icaA*, *cna* and *sdrE*), exfoliative toxins (*eta* and *etb*), toxic shock syndrome toxin (*tst*), hemolysin (*hlg*) and enterotoxins (*sea*, *seb*, *sec*, *sed* and *see*). The PCR was done in a thermocycler and the products were resolved, visualised and scored as described earlier. PCR-grade water was used as a negative control. The primer sequences, annealing temperatures, and reaction volume and conditions used are provided in Table S1.

Table 1
Prevalence of staphylococci isolates from various food samples based on phenotypic analysis.

Food type	Food Sample	Number of samples collected	Number of samples with staphylococci
Cooked	Banku	10	3 (30)
	Beans	15	15 (100)
	Fish	18	16 (88.89)
	Fufu	15	14 (93.33)
	Kenkey	16	6 (37.50)
	Pepper sauce	17	13 (76.47)
	Salad	14	14 (100)
	Soup	27	16 (59.26)
	Waakye	16	14 (87.50)
Total		148	111 (75)
Vegetable	Cabbage	12	10 (83.33)
	Carrot	14	14 (100)
	Tomato	13	8 (61.54)
Total		39	32 (82.05)
Fruit	Apple	11	8 (72.73)
	Banana	13	3 (23.08)
	Orange	12	7 (58.33)
	Pineapple	13	6 (46.15)
Total		49	24 (48.98)
Total		236	167 (70.76)

Cooked foods are local cuisines in Ghana. Values in brackets represent the percentage of the number of samples with staphylococci relative to the total number of each sample collected.

2.6. Detection of Staphylococcus species composition and antibiotic resistance and virulence determinants

The total composition of the staphylococcal species and their abundance were determined by loading the assembled sequences into the Kraken2 program [31]. Kranken2 uses exact k-mer matches for taxonomic classification. The antibiotic resistance genes and proteins together with associated antimicrobials were determined in the Comprehensive Antibiotic Resistance Database (CARD) [32]. The detection of major virulence factors in the *Staphylococcus* sequences was also performed in the MG-RAST [33] and the virulence factor database (VFDB) [34].

2.7. Statistical analysis

The proportions and frequencies of all variables were calculated using descriptive statistics in Excel. Categorical variables were compared using chi-square and Fischer's exact test at a 5 % ($p < 0.05$) statistical significance level. The relationship between the resistance profile of all antibiotics was tested using the Spearman correlation. Only strong associations ($-0.8 \geq r \geq 0.8$) were considered [35]. The statistical analyses were done using SPSS version 26 statistical software (SPSS Inc., USA).

Table 2
Prevalence of *Staphylococcus* species from various food samples based on PCR.

Food type	Food sample	Number of *Staphylococcus* species								
		S. aureus	*S. epidermidis*	*S. haemolyticus*	*S. pasteuri*	*S. saprophyticus*	*S. xylosus*	*S. warneri*	*S. caprae*	*S. capitis*
Cooked	Banku (3)	3 (100)	3 (100)	3 (100)	1 (33.33)	3 (100)	3 (100)	1 (33.33)	3 (100)	3 (100)
	Beans (15)	13 (86.67)	11 (73.33)	15 (100)	2 (13.33)	15 (100)	13 (86.67)	3 (20)	3 (20)	11 (73.33)
	Fish (16)	11 (68.75)	15 (93.75)	15 (93.75)	3 (18.75)	15 (93.75)	15 (93.75)	7 (43.75)	9 (56.25)	14 (87.50)
	Fufu (14)	13 (92.86)	12 (85.71)	14 (100)	2 (14.29)	14 (100)	14 (100)	6 (42.86)	7 (50)	11 (78.57)
	Kenkey (6)	5 (83.33)	5 (83.33)	6 (100)	1 (16.67)	6 (100)	6 (100)	3 (50)	2 (33.33)	5 (83.33)
	Pepper sauce (13)	12 (92.31)	12 (69.23)	13 (100)	3 (23.08)	13 (100)	13 (100)	6 (46.15)	7 (53.85)	8 (61.54)
	Salad (14)	11 (78.57)	10 (71.43)	14 (100)	2 (14.29)	13 (92.86)	12 (85.71)	6 (42.86)	5 (35.71)	11 (78.57)
	Soup (16)	10 (62.50)	11 (68.75)	15 (93.75)	3 (18.75)	14 (87.50)	12 (75)	3 (18.75)	5 (31.25)	11 (75)
	Waakye (14)	11 (78.57)	11 (78.57)	14 (100)	0 (0)	13 (92.86)	13 (92.86)	8 (57.14)	6 (42.86)	9 (64.29)
	Total (111)	89 (80.18)	87 (78.38)	109 (98.20)	17 (15.32)	106 (95.50)	101 (90.99)	43 (38.74)	47 (42.34)	84 (75.68)
Vegetable	Cabbage (10)	6 (60)	7 (70)	10 (100)	0 (0)	9 (90)	9 (90)	4 (40)	4 (40)	5 (50)
	Carrot (14)	6 (42.86)	7 (71.43)	14 (100)	2 (14.29)	14 (100)	13 (92.86)	5 (35.71)	6 (42.86)	7 (50)
	Tomato (8)	8 (100)	7 (87.50)	8 (100)	0 (0)	8 (100)	8 (100)	4 (50)	5 (62.50)	6 (75)
	Total (32)	20 (62.50)	24 (75)	32 (100)	2 (6.25)	31 (96.88)	30 (93.75)	13 (40.63)	15 (46.88)	18 (56.25)
Fruit	Apple (8)	6 (75)	6 (75)	8 (100)	2 (25)	8 (100)	7 (87.50)	3 (37.50)	4 (50)	5 (62.50)
	Banana (3)	2 (66.67)	2 (66.67)	3 (100)	1 (33.33)	2 (66.67)	2 (66.67)	1 (33.33)	1 (33.33)	2 (66.67)
	Orange (7)	6 (85.71)	5 (71.43)	7 (100)	1 (14.29)	6 (85.71)	7 (100)	3 (42.86)	5 (71.43)	5 (71.43)
	Pineapple (6)	6 (100)	5 (83.33)	6 (100)	1 (16.67)	6 (100)	6 (100)	2 (33.33)	3 (50)	5 (83.33)
	Total (24)	20 (83.33)	18 (75)	24 (100)	5 (20.83)	22 (91.67)	22 (91.67)	9 (37.50)	13 (54.17)	17 (70.83)
Total (167)		129 (77.25)	129 (77.25)	165 (98.80)	24 (14.37)	159 (95.21)	153 (91.62)	65 (38.92)	75 (44.91)	119 (71.26)

Cooked foods are local cuisines in Ghana, Food sample: Value in bracket represent the food samples with staphylococci isolates, Number of *Staphylococcus* species: Value in bracket represent the percentage of the *Staphylococcus* species present in each food sample.

3. Results

3.1. Prevalence of Staphylococcus species based on phenotypes and PCR

A total of 167 representing 70.76 % of the 236 food samples had staphylococci contamination (Table 1). A minimum of 3 out of the total samples collected for each food showed the presence of staphylococci. The prevalence of staphylococci was more common in vegetables (82.05 %) and cooked foods (75 %) than in fruits (48.98 %). The highest prevalence of staphylococci was found in beans and salad (100 %), carrots (100 %) and apples (72.73 %) for each food type. A high prevalence of the species (≥76.47 %) was recorded for five food samples (fish, fufu, pepper sauce, waakye and cabbage). On the contrary, tomato (61.54 %), banana (23.08 %) and banku (30 %) had the lowest percentage of staphylococci contamination among the food types. There was a statistically significant difference ($p < 0.05$) in the prevalence of staphylococcal isolates between food samples. Similarly, a statistically significant difference ($p < 0.05$) was observed in the prevalence of staphylococcal isolates between food types. All the staphylococci isolates had an amplicon for the *Staphylococcus* genus-specific *tuf* gene based on analysis with the TstaG422 and Tstag765 primers. The *Staphylococcus* species-specific PCR-based assay identified nine species (Table 2). *Staphylococcus haemolyticus* was the most prevalent (98.80 %) in the foods followed by *S. saprophyticus* (95.21 %) and *S. xylosus* (91.62 %). A high prevalence of *S. aureus* as well as *S. epidermidis* (77.25 %) was also shown in the foods. The prevalence of *S. capitis* was similarly high (71.26 %) whereas *S. caprae* (44.91 %), *S. warneri* (38.92 %) and *S. pasteuri* (14.37 %) were relatively low. Nevertheless, there was a total absence of *S. pasteuri* in three foods (waakye, cabbage and tomato). There was no statistically significant difference ($p > 0.05$) in the prevalence of the staphylococcal species between the food types as well as the food samples. However, the abundance of the staphylococci significantly differed ($p < 0.05$) between food samples.

3.2. Patterns of antimicrobial resistance of staphylococci based on phenotypes and PCR

The staphylococci from all the foods showed phenotypic resistance to tetracycline, gentamicin, levofloxacin, ciprofloxacin, amikacin and norfloxacin, and susceptibility to nitrofurantoin (Table S3). A strong association was found between nitrofurantoin and levofloxacin ($r = 0.863$, $p = 0.000$) and nitrofurantoin and amikacin resistance ($r = 0.827$, $p = 0.001$). There was a statistically significant difference ($p < 0.05$) in the antibiotic resistance pattern between the food types. For the five antibiotics without breakpoints, staphylococci from vegetables and cooked foods showed the highest inhibition zones for cefoperazone (20.75 mm), cefotaxime (15 mm), and piperacillin-tazobactam (14 mm), ceftriaxone (15.75 mm) and nalidixic acid (13 mm), respectively, compared with inhibition zones showed by staphylococci from fruits.

The PCR-based analysis indicated several antimicrobial resistance encoded genes in the staphylococci isolates, confirming the phenotypic assay (Table 3). Genes encoding for resistance to a broad spectrum of antimicrobials such as beta-lactams and fluoroquinolones (*acrA*), tetracycline (*tetA*) and streptomycin (*strA*) were the most common in the species (100 %). The species were also frequently (93.75 %) associated with *vanA* which is responsible for resistance to vancomycin. A high prevalence of staphylococci

Table 3
Distribution of antibiotic resistance genes in staphylococci isolates from various food samples by PCR.

Food type	Food sample	Number of antibiotic resistance gene									
		aac-(3)-1	acrA	ermA	ermB	mecA	strA	tetA	tetB	vanA	bla_{TEM}
Cooked	Banku (1)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
	Beans (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
	Fish (1)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)
	Fufu (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
	Kenkey (1)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
	Pepper sauce (1)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
	Salad (1)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	Soup (1)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)
	Waakye (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
	Total (9)	7 (77.78)	9 (100)	7 (77.78)	5 (55.56)	6 (66.67)	9 (100)	9 (100)	5 (55.56)	8 (88.89)	4 (44.44)
Vegetable	Cabbage (1)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
	Carrot (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
	Tomato (1)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)
	Total (3)	2 (66.67)	3 (100)	1 (33.33)	1 (33.33)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)	2 (66.67)
Fruit	Apple (1)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
	Banana (1)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
	Orange (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	Pineapple (1)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)
	Total (4)	3 (75)	4 (100)	2 (50)	2 (50)	2 (50)	4 (100)	4 (100)	1 (25)	4 (100)	3 (75)
Total (16)		12 (75)	16 (100)	10 (62.50)	8 (50)	11 (68.75)	16 (100)	16 (100)	6 (37.50)	15 (93.75)	9 (56.25)

Cooked foods are local cuisines in Ghana, Food sample: Value in bracket represent the food samples with staphylococci isolates, Number of antibiotic resistance gene: Value in bracket represent the percentage of the number of antibiotic resistance gene present in each food sample.

Table 4
Distribution of virulence genes in staphylococci isolates from various food samples as revealed by PCR.

Food type	Food sample	Number of virulence gene											
		cna	eta	etb	hlg	icaA	sdrE	tst	sea	seb	sec	see	sed
Cooked	Banku (1)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
	Beans (1)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
	Fish (1)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
	Fufu (1)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
	Kenkey (1)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)
	Pepper sauce (1)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)
	Salad (1)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)
	Soup (1)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
	Waakye (1)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
	Total (9)	2 (22.22)	9 (100)	6 (66.67)	2 (22.22)	3 (33.33)	3 (33.33)	6 (66.67)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Vegetable	Cabbage (1)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
	Carrot (1)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	Tomato (1)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
	Total (3)	0 (0)	2 (66.67)	2 (66.67)	1 (33.33)	1 (33.33)	1 (33.33)	2 (66.67)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Fruit	Apple (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
	Banana (1)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
	Orange (1)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
	Pineapple (1)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)
	Total (4)	0 (0)	1 (25)	2 (50)	1 (25)	1 (25)	1 (25)	2 (50)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total (16)		2 (12.50)	12 (75)	10 (62.50)	4 (25)	5 (31.25)	5 (31.25)	10 (62.50)	8 (50)	10 (62.50)	14 (87.50)	10 (62.50)	4 (25)

Cooked foods are local cuisines in Ghana, Food sample: Value in bracket represent all the staphylococci isolates from the food sample, Number of virulence gene: Value in bracket represent the percentage of the virulence gene present in the isolates from each food sample.

harboured *aac-(3)-1* (75 %), *mecA* (68.75 %) and *ermA* (62.50 %). These genes are related to resistance to gentamicin, methicillin and erythromycin, respectively.

The occurrence of beta-lactamase TEM (penicillins and cephalosporins resistance) was 56.25 %. The presence of *erm*(B) (50 %) and *tet*(B) (37.50 %) genes, conferring resistance to erythromycin and tetracycline, respectively, in the staphylococci were relatively low. There was no statistically significant difference ($p > 0.05$) in the prevalence of antibiotic resistance genes between the food samples and the food types.

3.3. Patterns of virulence factors of staphylococci based on PCR

A high number of staphylococci isolates harboured the exfoliative toxin genes *eta* (75 %) and *etb* (62.50 %), and toxic shock syndrome toxin 1 gene *tst* (62.50 %) based on PCR (Table 4). The prevalence of the intercellular adhesion gene *ica*A and the serine-aspartate repeat gene *sdrE* were similar in the staphylococci (31.25 %). The *Staphylococcus* species were also positive for *hlg* (25 %) and *cna* (12.50 %), which encode for γ-hemolysin and collagen-binding protein, respectively. The sec was the most common *Staphylococcus*

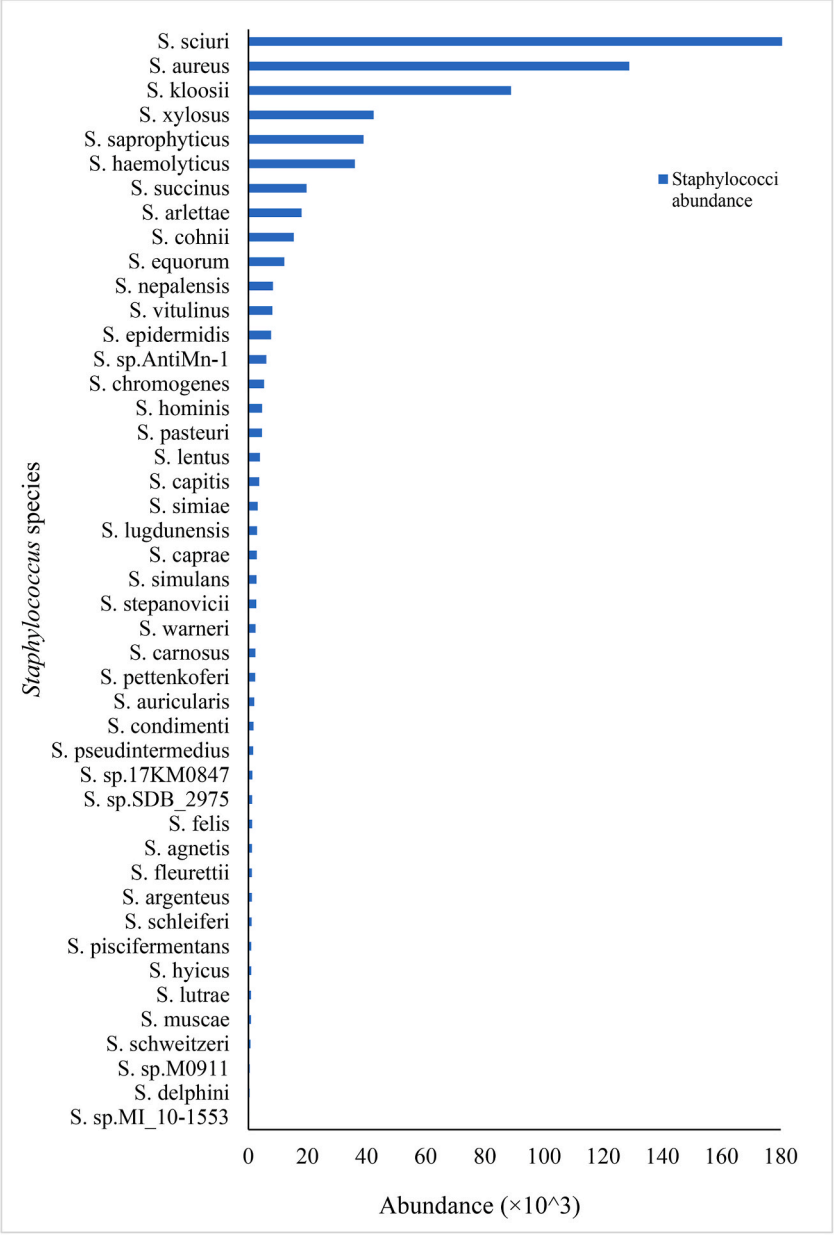

Fig. 1. Distribution of *Staphylococcus* species abundance based on WGS data.

enterotoxin (SE) gene in the isolates (87.5 %) followed by *seb* and *see* (62.50 %), *sea* (50 %) and *sed* (25 %) genes (Table 4). Statistically, a significant difference ($p < 0.05$) was seen in the prevalence of the virulence genes between the food samples. On the contrary, there was no significant difference (($p > 0.05$) in the virulence gene profile between food types.

3.4. Detection of Staphylococcus species based on WGS data

Genotypic identification based on the WGS data revealed 45 *Staphylococcus* species, comprising coagulase positive (CoP), coagulase-negative (CoN) and unspeciated staphylococci, and confirmed the phenotypic and PCR-based identification assay (Fig. 1). The species abundance ranged from 2.97×10^2 to 1630.13×10^3 and was evident for *Staphylococcus* sp MI 10–1553 and *Staphylococcus sciuri* (now *Mammaliicoccus sciuri* [36]), respectively. *S. aureus* showed a comparatively high abundance of 128.79×10^3. The presence

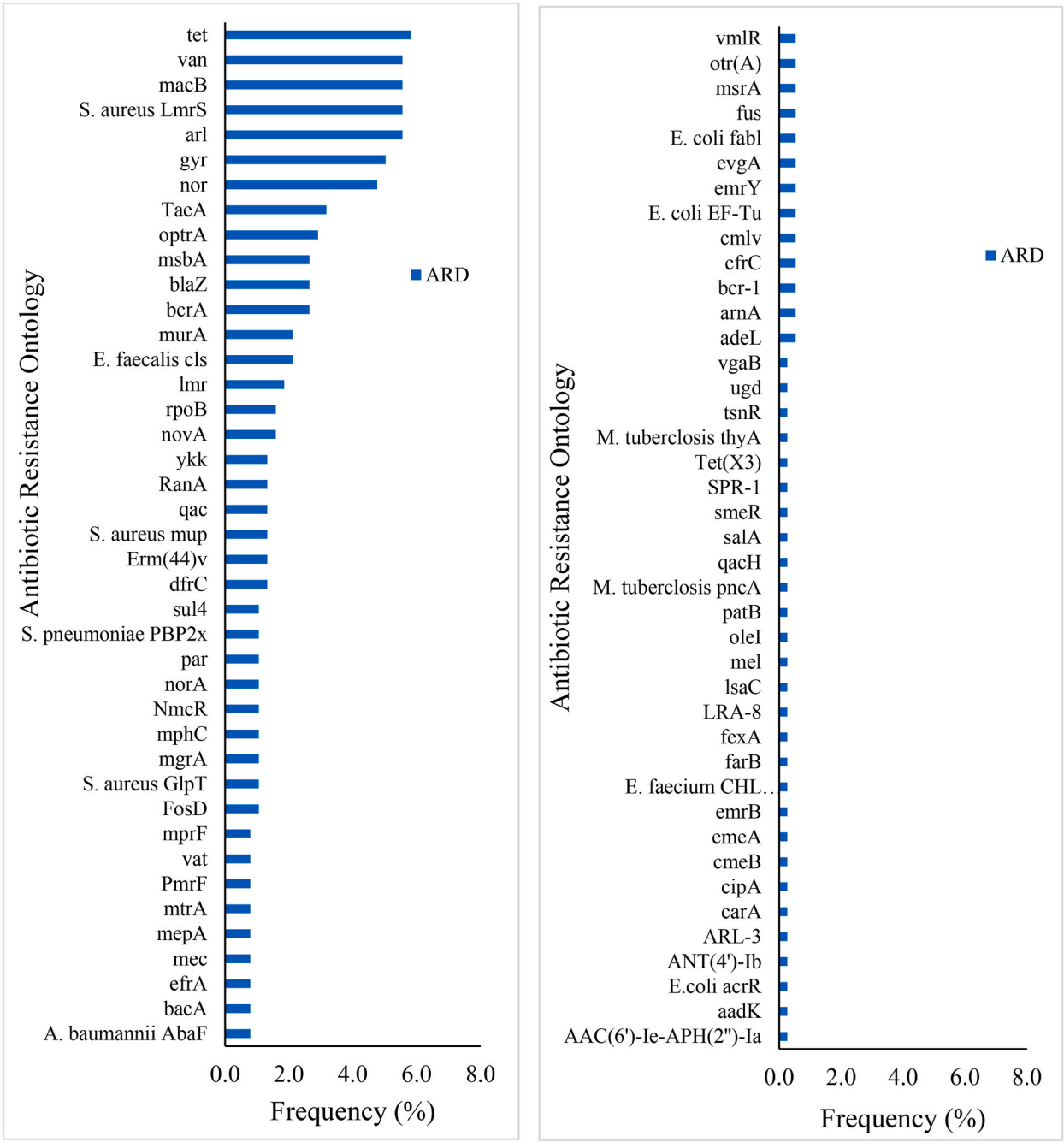

Fig. 2. Distribution of antibiotic resistance determinants (ARD) of *Staphylococcus* species based on WGS data.

and abundance of *S. kloosii* (88.85 × 10^3), *S. xylosus* (42.36 × 10^3), *S. saprophyticus* (38.95 × 10^3) and *S. haemolyticus* (36.04 × 10^3) were also detected. The food samples were also positive for *S. succinus* (19.73 × 10^3), *S. arlettae* (17.99 × 10^3), *S. cohnii* (15.39 × 10^3), *S. equorum* (12.19 × 10^3), *S. nepalensis* (8.36 × 10^3), *S. vitulinus* (8.12 × 10^3) and *S.* sp AntiMn-1 (6.07 × 10^3). *S. epidermidis* showed a higher abundance (7.72 × 10^3) compared with *S. chromogenes* (5.32 × 10^3), *S. hominis* (4.66 × 10^3), *S. pasteuri* (4.61 × 10^3), *S. capitis* (3.67 × 10^3), *S. lugdunensis* (2.96 × 10^3), *S. caprae* (2.86 × 10^3) and *S. warneri* (2.42 × 10^3).

3.5. Detection of genotypic determinants of resistance to antimicrobials based on CARD

A total of 125 antibiotic resistance determinants belonging to 82 groups of genes and proteins were detected from the staphylococci sequences based on the AMR gene family and drug class (Table S4). The majority of the determinants were associated with *S. aureus* (21.69 %), *S. saprophyticus* (18.78 %) and *S. epidermidis* (18.52 %). The presence of genes that encode antimicrobial resistance in *S. haemolyticus*, *S. ludgunensis*, *S. warneri*, *S. hominis* and *S. capitis* were 9.79, 7.94, 7.14, 5.56 and 4.50 %, respectively. The remaining staphylococcal species showed a low number of antibiotic-resistant determinants (less than 3 %).

The *tet* (tetracycline resistance) was the most prevalent gene (5.82 %) and commonly comprised *tetK* (18.18 %), *tetA* (58) and *tetT* (13.64 %), and *tet* (35) and *tetW* (9.09 %) (Fig. 2, Table S4). The *arl* (multidrug resistance) gene also occurred frequently (5.56 %) and consisted mainly of *arlS* (52.38 %) and *arlR* (47.62 %). The remaining determinants included *Staphylococcus aureus* LmrS (5.56 %), *macB* (5.56 %), *van* (5.56 %) (*vanHO*, *vanG*, *vanHF*, *vanKI*, *vanM* and *vanRM*), *gyr* (5.03 %) (*gyrA* and *gyrB*) and *nor* (4.76 %) (*S. aureus norA* and *norB*) encoding vancomycin (*van*), fluoroquinolones (*gyr*) and multidrug (*nor*, *macB*, *S. aureus* LmrS) resistance. The genes *taeA* (3.17 %), *blaZ* (2.65 %), *bcrA* (2.65 %), *msbA* (2.65 %) and *optrA* (2.91 %) were detected to confer resistance to pleuromutilin, beta-lactams, bacitracin, nitroimidazoles and multidrug, respectively. The *blaZ* frequently comprised *mec*C-type and PC1 beta-lactamase. The Erm (44)v (multidrug resistance) and *Streptococcus pneumoniae* penicillin-binding protein 2× (PBP2x) (amoxicillin resistance) were 1.32 % and 1.06 %, respectively.

Resistance was also detected for daptomycin (*cls*), fosfomycin (*murA*, *S. aureus* GlpT, FosD), lincosamides (*lmrB* and *lmrD*), novobiocin (*novA*), rifampicin (*rpoB*), trimethoprim (*dfrC*), mupirocin (*mupA* and *mupB*), fluoroquinolones (*qacA* and *qacB*), aminoglycosides (RanA) and multidrug (*ykkC*, *ykkD* and *mgrA*). The frequencies of 21.4 % of the determinants were less frequent (less than 1 %). These included the *Acinetobacter baumannii* AbaF protein, *vat* (*vatB* and *vatE*), *arnA*, *bcr-1*, *tsnR*, *fus* (*fusA* and *fusD*), *Mycobacterium tuberculosis thyA*, ARL-3 and aminoglycoside acetyltransferase AAC (6′)-Ie-APH(2″)-Ia, aminoglycoside nucleotidyltransferase ANT (4′)-Ib and *aadK* which offer resistance to fosfomycin, streptogramins, polymyxin, bicyclomycin, thiostrepton, fusidic acid, *para*-aminosalicylic acid, beta-lactams and aminoglycosides, respectively. The *mprF*, *E. coli fabI* and NmcR determinants are associated with defensin (*Bacillus subtilis mprF*) and daptomycin (*S. aureus mprF*), isoniazid and triclosan and beta-lactam resistance, respectively. The *mec* (methicillin resistance) gene constituted 0.8 % and was attributed to *mecD*, *mecI* and *mecR1*.

3.6. Detection of genotypic drug resistance and mechanism

The resistance to multiple antimicrobials was predominant (30.42 %) and ranged from two drug classes (11.30 %) (tetracyclines and glycylcyclines, isoniazid (antitubercular agents) and triclosan) to eight drug classes (4.35 %) (fluoroquinolones, cephalosporins, glycylcyclines, penams, tetracyclines, rifamycins, phenicols and triclosan) (Figs. 3 and 4). Multidrug resistance (resistance to antimicrobials from three or more classes) accounted for 86.96 % of the multiresistance. Resistance to four drug classes (carbapenems, cephalosporins, cephamycins and penams) was the most frequent (30.43 %), followed by five drug classes (19.13 %) (macrolides, aminoglycosides, oxazolidinones, diaminopyrimidines and phenicols), seven drug classes (17.39 %) (macrolides, lincosamides, streptogramins, tetracyclines, oxazolidinones, phenicols and pleuromutilins) and three drug classes (15.65 %) (aminoglycosides,

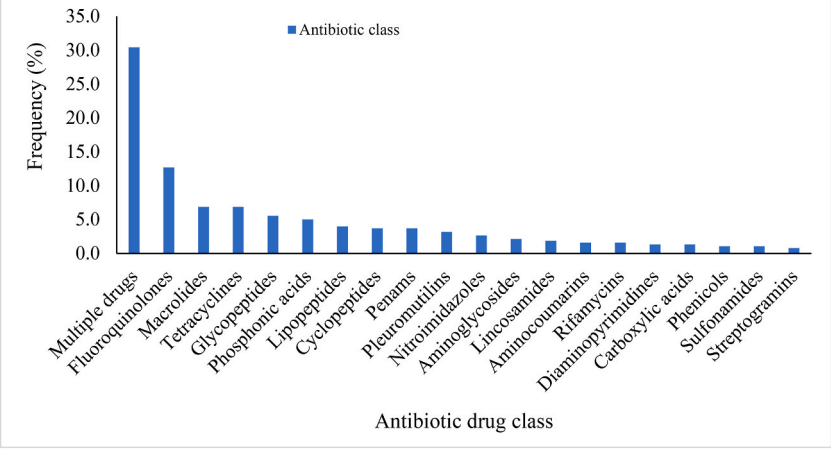

Fig. 3. Antibiotic classes of resistance determinants of *Staphylococcus* species based on WGS data.

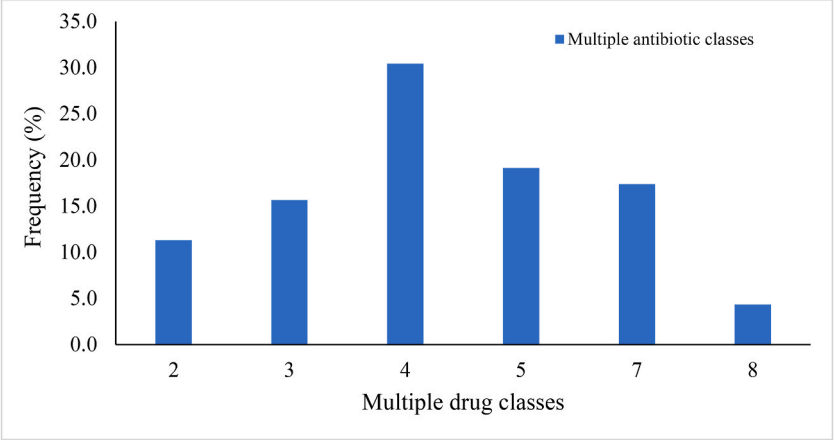

Fig. 4. Prevalence of determinants conferring resistance to multiple antimicrobials based on WGS data.

tetracyclines and phenicols) (Fig. 4).

The encoding determinants were predominantly proteins of the major facilitator superfamily (MFS) (40 %), ATP-binding cassette F (ABC-F) (17.39 %), resistance-nodulation-cell division (RND) (6.96 %) and small multidrug resistance (SMR) (5.22 %) (Fig. S1). The Erm 23S ribosomal RNA methyltransferase (4.35 %), multidrug and toxic compound extrusion (MATE) (3.48 %), NmcA beta-lactamase (3.48 %) and ABC (2.61 %) were also present. Fluoroquinolones (moxifloxacin) alone was the most frequent drug class that the majority of the isolates were susceptible (12.70 %) (Fig. 3). This was followed by macrolides (*mphC*) and tetracyclines (*emrY*) (6.88 %), glycopeptides (vancomycin) (5.56 %) and phosphonic acids (fosfomycin) (5.03 %). Resistance to lipopeptides (daptomycin) (3.97 %), cyclopeptides (bacitracin) and penams (methicillin) (3.70 %), pleuromutilins (tiamulin) (3.17 %), nitroimidazoles (*msbA*) (2.65 %), aminoglycosides (*aadK*, ANT (4′)) (2.12 %) and lincosamides (*lmrB*) (1.85 %) were also detected. Resistance to aminocoumarins (novobiocin) and rifamycins (rifampicin) was at 1.59 % each.

Low resistance (between 0.26 % and 1.32 %) was similarly reported for 11 antimicrobials. This included diaminopyrimidines (trimethoprim), carboxylic acids (mupirocin), phenicols (chloramphenicol), sulfonamides, streptogramins, bicyclomycin, elfamycins (kirromycin), fusidanes (fusidic acid) and antitubercular agents (pyrazinamide). Carbapenems and antimicrobial lipids were the least detected and were encoded by SPR beta-lactamase and antibacterial free fatty acids, respectively. The antibiotic resistance mechanisms of the determinants were mostly efflux pump (50.53 %) whilst a combination of target alteration and efflux pump (about 0.26 %) was the least resistance mechanism (Fig. 5). Target alteration, inactivation, target protection and target replacement were 26.72 %, 10.05 %, 7.67 % and 3.17 %, respectively. Target alteration plus replacement was 1.59 %.

3.7. Detection of virulence factor determinants

The major virulence factors of the *Staphylococcus* species were further identified from the sequences of the species (Fig. 6). A total of

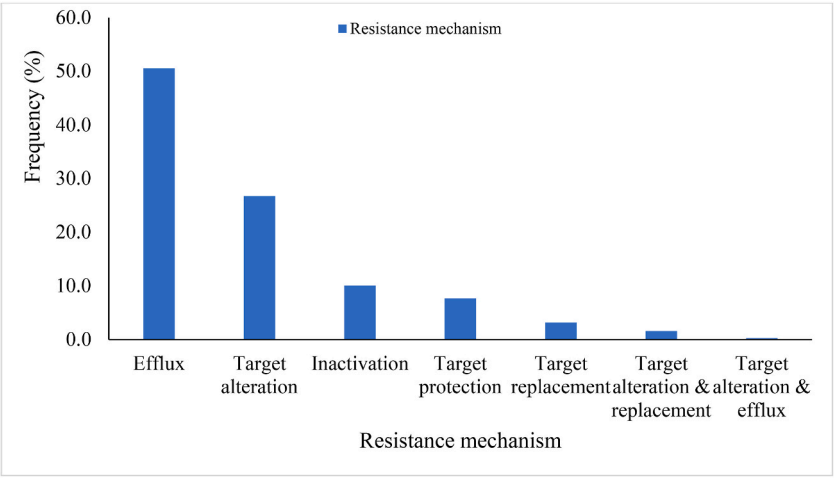

Fig. 5. Antibiotic resistance mechanisms of *Staphylococcus* species based on WGS data.

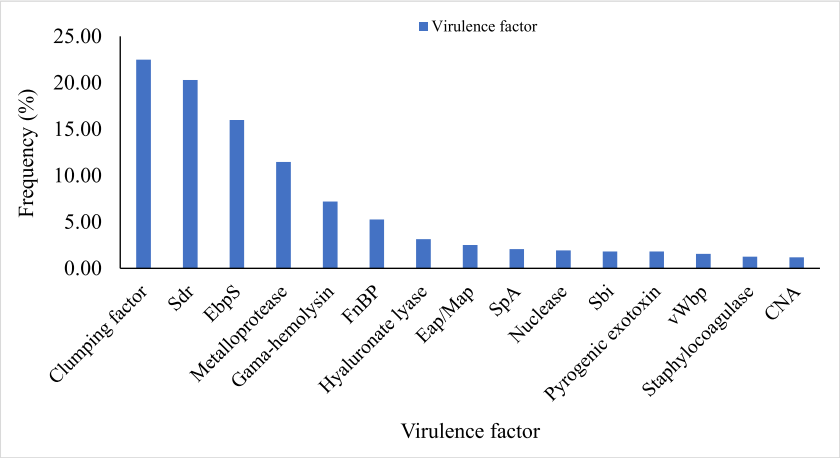

Fig. 6. Virulence factors of Staphylococcus species based on WGS data.

15 virulence factors were recorded and commonly comprised clumping factor (*clf*) (22.49 %), serine-aspartate repeat, Sdr (*sdr*) (20.30 %), elastin binding protein, Ebps (*ebp*) (15.98 %) and metalloprotease (11.47 %). The *sdrC* and *sdrD* were the most frequent genes (61.11 % and 19.75 %, respectively) of Sdr. The major virulence factors similarly included γ-hemolysins (7.21 %), fibronectin-binding proteins, FnBPs (*fnbA* and *fnbB*) (5.26 %), hyaluronate lyase (3.13 %), extracellular adherence protein/MHC analogous protein of broad specificity (Eap/Map) (2.51 %), and staphylococcal protein A (SpA) (2.07 %). Nuclease, staphylococcal binder of immunoglobulins (Sbi), pyrogenic exotoxin and secreted von Willebrand factor binding protein (vWbp) were also present with frequencies ranging from 1.57 % to 1.94 %. The remaining major virulence factors recorded for the species were staphylocoagulase (1.25 %) and collagen-binding protein (CNA) (1.19 %) with related genes *coa* and *cna*, respectively.

4. Discussion

The high number of 45 staphylococcal species identified supports the idea of significant species diversity of staphylococci in foods and reinforces the notion of diverse *Staphylococcus* species in Africa [16]. The most abundant staphylococcal species, *S. sciuri* (*M. sciuri*), has a wide host range and adapts to different habitats [37,38]. The staphylococci identified in this study are clinically relevant and known to cause a variety of community-acquired, farm-acquired and nosocomial-acquired infections such as pneumonia and meningitis in humans [16,39–41]. The observation made in the abundance of these pathogens underscores the need for pragmatic measures including the creation and enforcement of awareness and personal hygiene to mitigate the occurrence of *Staphylococcus* infections.

Staphylococci are increasingly associated with antimicrobial multiresistance mechanisms [42]. The staphylococci identified in this study showed resistance to tetracycline, levofloxacin, ciprofloxacin, norfloxacin, gentamicin and amikacin. This confirms the findings of earlier studies that reported a high antibiotic resistance profile in staphylococci [40,43,44]. The susceptibility of all the staphylococci isolates to nitrofurantoin may denote its potency in treating *Staphylococcus* infections. Nitrofurantoin was similarly detected as the most active antibiotic against strains of *Staphylococcus* isolates from cheese as well as patients with urogenital infections [45,46]. The PCR and WGS assays confirmed the phenotypic responses of the species to the antibiotics and affirmed a critical therapeutic implication to human health in the face of the ever-increasing shortage of effective antibiotics against staphylococci [47,48]. The pattern of antibiotic resistance observed in *S. aureus*, *S. saprophyticus* and *S. epidermidis* in this study was consistent with previously reported findings in clinical isolates of these species from the oral cavity [40,44,49]. The resistance determinants identified such as *blaZ*, *tetL*, *tetK*, *mecA*, *ermA*, *ermB*, *rpoB* and *gyrA* have been reported in staphylococci [50].

The *optrA* and *cfrC* confer MDR and commonly coexist in MRSA with a high potential for dissemination among gram-positive bacteria [51–53]. Tigecycline is the last line of defence against severe infection and is threatened by the increasing occurrence of *tet* (X3) genes in animals, meat for consumption and humans [54–56]. The production of PBP2a in staphylococci is regulated by the *blaZ-blaI-blaR1* and *mecA-mecI-mecRI* systems and confers resistance to methicillin and all beta-lactams [57–59]. The resistance of the staphylococci isolates to tetracyclines, penicillins, sulfonamides, trimethoprim, macrolides, lincosamides, fluoroquinolones, aminoglycosides, pleuromutulins and polymyxins could be associated with the frequent use of these antibiotic classes in food-producing animals and plant [60]. The multidrug resistance pattern ranged from three to eight drug classes. The high prevalence of staphylococci resistance to fluoroquinolones is influenced by the high occurrence of efflux pumps and target alteration resistance mechanisms in the species [61]. The efflux pump functions to maintain low-intracellular concentrations of antibiotics by exporting the antibiotic agents from bacteria cells before reaching their targets [62,63]. The MFS, ABC, RND, SMR and MATE proteins detected are the main families of efflux pumps in bacteria in terms of energy source and structure [64]. The findings suggest a strong tendency for XDR in *Staphylococcus* species and affirm the rapid increase in the prevalence of XDR in bacterial species [65,66].

The study revealed a high prevalence of major virulence genes in the staphylococcal species which were commonly associated with

adherence (*clf*, *ebpS*), exoenzyme (*coa*, *vWbp*), immune evasion (Sbi, *spA*, *nuc*) and toxin production (*hlg*, *eta*). The staphylococcal isolates also had the toxinogenic ability to cause food poisoning (*sea* to *see*), stimulate the epithelial secretion of proinflammatory cytokines to induce toxic shock syndrome with high mortality (*tst*) and destroy desmosomal cell attachments to cause scalded skin syndrome (*eta*, *etb*) [41,67]. The high frequency of the clumping factor signifies the abundance of the gene in the species. The presence of the gene, as well as the genes for fibronectin-binding proteins, elastin-binding proteins and collagen-binding proteins, is crucial for the staphylococcal species to bind, colonise and invade host cells [68]. The presence of the *icaA* gene together with sec and *hlg* confers enhanced pathogenicity in staphylococci [16]. The putative roles of these virulence factors in the antimicrobial resistance of staphylococci have been documented and explored as targets for novel therapeutic agents against staphylococcal infections [69–71].

5. Conclusions

Food samples had a high level of staphylococcal contamination, which varied in composition. The identified species comprised CoNs and CoPs staphylococcal species and are commonly implicated in various animal and human infections. The staphylococcal species were multidrug-resistant to three or more drug classes. Staphylococci were frequently associated with the *tet* genes, efflux pump proteins and susceptibility to fluoroquinolones. The presence of adhesins, exoproteins and pyrogenic toxin superantigens was commonly detected. Interestingly, these isolates were susceptible to nitrofurantoin. The next-generation sequencing technique revealed better insight into the diversity of staphylococci and their antimicrobial resistance and virulence genes. The prevalence, phenotypic and genetic multiresistance of *Staphylococcus* species to antimicrobials remains a critical health concern and there is a need for routine monitoring of staphylococcal levels in foods to ensure food and human safety. This study was limited to the assessment of antibiotic resistance and virulence patterns in staphylococcal isolates. A few primers were used for PCR analysis. A tracer study is required to identify the possible sources of antimicrobial-resistant staphylococci in food.

Funding

This work was financially supported by the Directorate of Research, Innovation and Consultancy of the University of Cape Coast under the grant code RSG/GRP/CANS/2020/107.

Ethics approval

This study was approved by the University of Cape Coast Institutional Review Board (UCCIRB/EXT/2020/46).

Availability of data and materials

The datasets used during the current study are available from the corresponding author upon request. The WGS data are publicly available in the MG-RAST database with accession numbers 4963321.3 and 4963322.3.

CRediT authorship contribution statement

Daniel Sakyi Agyirifo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Theophilus Abonyi Mensah:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Andrews Senyenam Yao Senya:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Alphonse Hounkpe:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Cindy Deladem Dornyoh:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Emmanuel Plas Otwe:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Directorate of Research, Innovation and Consultancy of the University of Cape Coast for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21584.

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