1. Introduction

Gram-positive coccal bacteria are normally found as a part of the human upper respiratory tract (URT) microflora [1]. However, due to several factors like age, immunity, social and economic factors, recent drug treatments and climate changes, those opportunistic colonizers may turn into serious pathogenic microorganisms causing severe diseases that may lead to human death [2,3].

*Staphylococcus* species are considered to be opportunistic pathogens mainly of the skin and nasopharynx as their most favourable reservoirs [4,5]. Among *Staphylococcus* species, *Staphylococcus aureus* is believed to be significantly responsible for causing severe respiratory infections in children from various regions of the world [6–8]. They can also be carried asymptomatically with varying rates in the nasopharynx of some children [9–11].

Although there are many antibacterial agents used against *S. aureus* infection still, the bacterium managed to develop several mechanisms such as the methicillin resistance mechanism to counteract them [12–14]. There has been an alarming increasing rate of health care, community and livestock-associated infections linked to methicillin-resistant *S. aureus* (MRSA) worldwide [8, 13–15]. Such huge increase in MRSA strains isolation is mostly related to the massive administration of antibiotics in medical settings, as well as in food manufacture and animal caring facilities for several decades [16].

Methicillin resistance is associated with a specific type protein that alters penicillin binding sites (PBP2a) on bacterial cell wall hence, reducing their affinity to bind β-lactam antibiotics [17]. Such resistance to methicillin is determined by the presence of mecA gene on a genetically mobile element known as staphylococci cassette chromosome mec (SCCmec) which carries several functional genes, all together play an important role in staphylococci pathogenesis [18].

Customary culture based methods is still considered as the standard conventional microbiological procedure used for the isolation and detection of *S. aureus* showing resistance to methicillin, but at the expense of time. Since such technique involves the isolation of *S. aureus* on selective media, followed by methicillin resistance confirmation using drug testing assay, which could take all together up to 48 or 72 h [19,20]. Molecular based techniques were developed to reduce time and increase the sensitivity of detecting MRSA. [21]. Multiplex PCR assay has been recently established as
a suitable substitute for the rapid detection of MRSA, decreasing the examination time to approximately 18 h [22–24].

The 16S rRNA gene of staphylococci was found to contain highly conserved DNA sequences at the genus level hence, separating them for other bacterial genera. Since then, several primers have been designed for the detection of staphylococci using their 16S rRNA gene. Staph-756F and Staph-750R 16S rRNA gene primer pairs were previously proven to be one of the most successful in staphylococci identification [25–28].

Additionally, nuclease gene (nuc) was reported as a specific target gene for detecting S. aureus [23,29]. Also, methicillin-resistance mecA gene has been designated as a molecular marker gene extensively used for MRSA detection and typing [30–32]. Therefore, the combined use of all 16S rRNA, nuc and mecA gene primers in a single multiplex PCR assay could provide a reliable, rapid and accurate detection of MRSA.

This study aimed to screen boys for asymptomatic carriage of potential bacterial pathogens such as methicillin-resistant S. aureus and detect its prevalence, taking into consideration the geographical bio-map that shows the levels of microbial pathogens in several districts. Furthermore, to compare the results obtained from traditional culture method which is considered as the traditional base for the detection of MRSA with that of the multiplex PCR method used for the amplification of the genes encoding the 16S rRNA and nuclease (nuc), as well as (mecA) gene for methicillin resistance determination.

2. Materials and methods

2.1. Population under study

The current study was conducted during autumn and winter seasons of 2016, for a period of 3 months (from beginning of October till end of December). The study population included double sets of school boys comprising 405 elementary students of age group 7–12 and 10–12 years. This total number of 405 school boys was selected from 8 governmental schools at different geographic positions, all located within Al-Madinah city, Kingdom of Saudi Arabia (Table 1 and Figure 1). Students suffering from any respiratory infection symptoms or were taking antibiotic treatment during the period of sampling were excluded from the study. Verified consent was attained from the principals of the chosen schools, parents and participating students before sample collection.

2.2. Sample collection and isolation

The specimens were collected as a single nasopharyngeal swap taken from each participant using calcium alginate coated swabs. Swaps were stored in transport media (HiMedia, Lab., Mumbai, India) that was labelled with a code number and relevant information including age and location. All specimens were directly kept on dry ice during transportation and were taken to the laboratory within 1–2 h. In the laboratory each swap was streaked the same day on nutrient agar and blood agar (5% sheep blood) plates and incubated at 37°C overnight, to be examined for culture characteristics and type of blood hemolysis. Bacterial colonies showing blood hemolysis were sub-cultured to obtain pure growth. This was followed by basic microbiological identification based on catalase and coagulase properties, cultural characteristics and colour on mannitol salt agar growth medium and cell morphology with Gram staining. Staphylococci have the unique ability to grow on the high salt containing mannitol salt agar media where other bacteria fail to grow. Coagulase-positive Staphylococcus aureus ferment mannitol producing yellow coloured colonies surrounded by yellow zones while other coagulase-negative Staphylococci are usually mannitol non-fermenters producing pink to red colonies surrounded by red-purple zones [19].

2.3. DNA extraction

The identity of the isolates was confirmed using molecular approaches as follows: bacterial colonies showing hemolytic activity were inoculated in 10 ml Luria–Bertani (LB) media (Difco, USA) and kept overnight at 37°C for genomic DNA extraction. After incubation, genomic DNA was extracted using DNA purification kit (Promega, USA) as recommended in the protocol. An acceptable DNA concentration (~5 µg/ml) and purity (A260/A280 ratio of 1.8) was determined by UV–visible spectrophotometer (Jenway, UK).

2.4. Amplification of 16S rRNA genes

The amplification of 16S rRNA genes was carried out using universal 8F forward primer and 1492R reverse primer [33], in an automated thermal cycler (Applied Biosystems, USA). PCR amplification cycles were conducted according the following conditions: initial denaturation at 94°C for 5 min, then 30 cycles of denaturation at
94°C for 45 s, 54°C annealing for 45 s, 72°C extension for 60 s followed by a final extension cycle for 7 min at 72°C. Each PCR reaction (New England Biolabs, USA) was performed in a 50-μl volume containing template DNA (200 ng/μl), forward and reverse primers (0.1 µM), dNTP mix (each at 10 mM), PCR buffer pH 8.3 (50 mM KCl, 10 mM Tris HCl and 1.5 mM MgCl2), Taq polymerase (1.25 unit/50 μl). After PCR program was completed the amplified products were electrophoresed along with 1 kb DNA marker (Invitrogen, USA) on a 1% agarose gel stained with 0.5 μg/ml ethidium bromide. After electrophoresis the resulting DNA fragments were observed under UV transilluminator.

2.5. Restriction enzyme analysis of the amplified 16S rDNA

To detect sequence variations among characterized isolates, restriction analysis was carried out on each of the amplified ribosomal DNA PCR product. The two double digestion reactions with both Hae III and Taq I four base cutter restriction enzymes in the first and EcoR I and BamH I six base cutters in the second (New England Biolabs, USA), were conducted for 3 h at 37°C, then digestion was stopped for both enzymes by incubating the reactions at 80°C for 20 min. Restriction fragments were separated on 3% agarose gel and observed with the gel documentation machine (Gel Doc XR, bio-rad, UK). Generated restriction patterns were analysed with PyElph 1.3 software using the clustering algorithm of un-weighted pair group method of arithmetic average (UPGMA) and dice pair-wise coefficient of similarity. Bacterial isolates with similar pattern were grouped together.

2.6. DNA sequence analysis and phylogeny

Representative isolates with different ARDRA pattern were selected for DNA sequencing using Big-Dye chain terminator cycle sequencing [34] at Macrogen Inc., Seoul, Korea. FinchTV application (Geospiza, Inc.) was used for viewing and editing DNA sequencing data. The sequences of the assembled 16S rRNA were used in a blast search analysis against the nucleotide database (http://www.ncbi.nlm.nih.gov/BLASTP/) to identify the closest similarities to the DNA sequences published in the GenBank database. Sequences showing high similarity (> 97% identity) were retrieved from GenBank and used along with obtained sequences to construct a phylogenetic tree. Clustal W1.83 XP was used for sequence alignments [35] and neighbour-joining method [36] for the construction of phylogenetic trees with MEGA6 software [37]. Sequences of the 16S rRNA genes from this study were submitted in GenBank (DDBJ) under accession numbers LC529200–LC529202.

2.7. Testing for methicillin resistance

To determine S. aureus isolates resistance against methicillin, Kirby–Bauer in vitro disc diffusion assay was performed with 1 µg oxacillin discs on Mueller–Hinton plates as instructed by the Institute of Clinical and Laboratory Standards. After 24 h of incubation, the presence or absence of inhibition zones was recorded and interpreted in accordance with CLSI (2015) guidelines [38].

2.8. Conventional multiplex PCR

Multiplex PCR assay using genomic DNA of Staphylococcus isolates as template was executed to target the 16S rDNA sequences specific to genus Staphylococcus, the S. aureus species specific gene (nuc) and the methicillin resistance gene (mecA). All primers used in this study are presented in Table 2. Multiplex PCR reaction was performed in a 50-μl volume containing template DNA (200 ng/μl), (1.25 unit/50 μl) of Taq DNA polymerase, (0.2 μM) of each forward and reverse primers used in the reaction, dNTP mix (each at 0.4 mM) and the formally indicated PCR buffer (with MgCl2...
Table 2. Nucleotide sequences of the primers used in the multiplex PCR for the detection of 16S rRNA, mecA and nuc genes.

| Target gene | Primer | Oligonucleotide sequences (5′−3′) | Amplicon size (bp) |
|-------------|--------|----------------------------------|-------------------|
| 16S rRNA    | Staph-756 F | 5′-AAC TCT GTT ATT AGG GAA GAA CA-3 | 756               |
|             | Staph-750 R | 5′-CCA CCT TCC TCC GTT TGG TCA CC-3 |                  |
| nucase      | nuc F   | 5′-GGG ATT GAT GAT GAT GAT GGT-3′ | 279               |
|             | nuc R   | 5′-AGG CAA GCC TTG AGC AAT TAA AGC-3′ |                  |
| mecA        | mecA-F  | 5′- GTA GAA ATG CTA GAA CTA CGG ATA A-3 | 533               |
|             | mecA-R  | 5′- GCC ATT CCA CAT TGT TTC GTG CTA A-3 |                  |

concentration adjusted to 2.5 mM). The amplification profile was conducted as described in previous reports [23,39], accordingly the conditions were as follows: first denature step for 5 min at 94°C, followed by 10 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s and extension at 72°C for 1 min, then another 25 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 75 s and finally an extension step of 10 min at 72°C. After PCR program was completed amplicons were loaded into 2% agarose gel stained with 0.5 μg/ml ethidium bromide and electrophoresed along with 100 bp molecular weight marker. The resulting DNA fragments were observed and photographed using gel documentation machine.

2.9. Ethical agreement

The research plane was approved by the Ethics Committee of the Taibah University and the ministry of education, Kingdom of Saudi Arabia. Consent forms were provided to each participating student and his parent or guardian explaining all the relevant details regarding the study through the school staff. Investigators also made sure that students knew they can stop or withdraw at any time. All students’ information and test results will be confidentially kept.

3. Results

3.1. Isolation of bacteria from nasopharyngeal cavity of elementary school boys

This study included a total of 405 participating male students of two age groups, 134 (33%) belonged to first group 7–9 years of age (average = 7.8 ± 1.19 years, mean of 8 years) and 271 (67%) belong to the second group 10–12 years (average = 11.12 ± 1.1 years, mean of = 11 years) from eight schools located within Al-Madinah city. A total of 138 catalase positive bacterial isolates identified under microscope as Gram-positive cocci obtained from all 405 participating student showed blood hemolysis on sheep blood agar plates giving an overall carriage rate of 34.1%. Isolates were grouped according to their culture characteristics, colony morphology and colour, coagulate reaction and blood hemolysis into 12 distinct groups.

3.2. Characterization of isolates

Representative isolates from each phenotypic group were subjected to restriction enzyme analysis via Amplified Ribosomal DNA Restriction Analysis (ARDRA) to reveal genotypic characteristics. The amplification of 16S rRNA gene by PCR was followed by restriction analysis in two double digestion reaction using both HaeIII and TaqI in the first reaction and EcoRI and BamHI in the second. The digestion of the 16S rDNA region generated a complex pattern of bands in which each sample yielded from two to eight major fragments ranging between 100–700 bp. The generated fragments displayed different pattern types and revealed an obvious polymorphism among the bacterial isolates. Cluster analysis using UPGMA algorithm revealed four distinct patterns among all 138 bacteria isolates (Figure 2). The majority of the bacterial isolates (110) belonging to five phenotypic groups displayed a unique ARDRA pattern I. All of 110 isolates showed positive growth of yellow colonies typical of staphylococci on mannitol salt agar plates. The remaining bacterial isolates showed another three different and characteristic ARDRA patterns and therefore clustered at different clades: cluster II (12 isolates), cluster III (7 isolates) and cluster IV (9 isolates).

3.3. Molecular typing and phylogenetic affiliation

Representative isolates from each ARDRA cluster were subjected to sequence-based bacterial typing via 16S rRNA gene sequence analysis. DNA sequencing of the 16S rRNA gene provided a reliable identification of the isolates after a BLAST search against the non-redundant nucleotide database hosted by the GenBank. Analysis of almost whole 16S rRNA genes revealed four specific species, namely, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus simulans and Staphylococcus hominis. The detailed sequence similarity results are all represented in Table 3. Phylogenetic studies confirmed the association of the identified genotypes to S. aureus, S. epidermidis, S. simulans and S. hominis clusters as observed through clustering of each species to its matching group. Based on 16S rRNA gene sequences analysis, the constructed phylogenetic tree successfully illustrated the affiliation between the selected isolates and their representative closest matches (Figure 3).
Figure 2. ARDRA of representative isolates from different phenotype groups after double digestion with (HaeIII/TaqI) and (EcoRI/BamHI) and electrophoreses on 3% agarose gel against 100 bp DNA ladder (Invitrogen, CA, USA). Restriction patterns were analysed by PyElph 1.3 software and dendrogram was generated using UPGMA algorithm and Dice (SD) pairwise similarity coefficient. Clusters were defined at SD > 70% similarity level.

Table 3. BLAST search results of the representative isolates with their best matched species from the GenBank database including their sequences accession numbers and similarities.

| ARDRA pattern | No. of isolates | Code   | Identity                      | Accession No. | Similarity (%) | Accession No. |
|---------------|----------------|--------|-------------------------------|---------------|----------------|---------------|
| I             | 110            | M-SAW2 | *Staphylococcus aureus* NBRC100910 | NR-113956     | 100            | LC529200      |
| II            | 12             | M-SAW1 | *Staphylococcus epidermidis* NBRC | NR-113957     | 99             | LC529199      |
| III           | 7              | M-SAW3 | *Staphylococcus simulans* MK148 | NR-036906     | 99             | LC529201      |
| IV            | 9              | M-SAW4 | *Staphylococcus hominis* GTC1228 | NR-041323     | 99             | LC529202      |

4Number of isolates in the corresponding ARDRA profile.
5Code for a representative isolate from each group.
6GeneBank sequence accession numbers of most closely related sequences.
7GeneBank sequence accession numbers of selected strains.

3.4. Prevalence of *Staphylococcus* spp. in elementary school boys

The prevalence of potential asymptomatic 138 *Staphylococcus* spp. in the nasopharyngeal cavity of elementary school boys was investigated. Of all isolates, *Staphylococcus aureus* accounted for 110 (carriage rate of 27.2%), followed by *Staphylococcus epidermidis* accounting for 12 (carriage rate of 3%), *Staphylococcus hominis* for 9 (carriage rate of 2.2%) and finally *Staphylococcus simulans* for 7 (carriage rate of 1.7%) (Figure 4A). *Staphylococcus* spp. were distributed in nasopharyngeal cavity of elementary school boys with different age groups by varying degrees. *S. aureus* was the predominant strain, however, *S. epidermidis*, *S. simulans* and *S. hominis* were significantly present especially in students from schools located at the northern area of Al-Madinah region. It is also worthy to mention that *S. simulans* was absent in school boys from the western area compared with the remaining six schools (Figure 4B). This study also revealed that the number of *Staphylococcus* spp. was greater in participants of age group 7–9 years compared to age group of 10–12 years (Figure 4C). Also, the prevalence of the bacterial species identified in this study (*S. aureus*, *S. epidermidis*, *S. hominis* and *S. simulans*) was higher in students with age group 7–9 years compared with age group of 10–12 (Table 4).

3.5. Identification of isolates showing resistant to methicillin

For the detection of methicillin resistance among isolated strains along with the validation of DNA sequencing results, all 138 bacterial isolates were examined using multiplex PCR method. Results indicated that each of the primer pairs used in the reaction amplified the single-target gene amplicons that corresponds to the expected sizes (Figure 5A). The nuc and 16S rRNA genes were only amplified from the 110 *S. aureus* isolates by multiplex PCR. These results corresponded precisely with those from DNA sequencing, proving the reliability of the multiplex PCR. The methicillin resistance mecA gene was detected in 22 (20%) *S. aureus*
Figure 3. Neighbour-joining phylogenetic tree showing the relationship between representative genotypes with different ARDRA patterns and closest matches from GenBank database. The bar represents 0.01 substitutions per site, bootstrap values (n = 1000) are displayed. *Bacillus cereus* ATCC14579 (NR−074540) was used for outgrouping.

Table 4. Age distribution of study population and bacteria isolated in different age groups.

| School Location | No. of participants (age group/years) | No. of hemolytic colonies | Distribution of bacterial isolates among age group/years* |
|----------------|--------------------------------------|---------------------------|----------------------------------------------------------|
|                | 7–9 years | 10–12 years | 7–9 years | 10–12 years | Sa | Se | Sh | Ss | Sa | Se | Sh | Ss |
| North          |           |             |           |             |     |     |     |     |     |     |     |     |     |
| N1             | 7         | 18          | 5         | 4           | 3   | 1   | 1   | 1   | 3   | 1   | –   | –   |
| N2             | 22        | 38          | 13        | 9           | 9   | 2   | 1   | 1   | 7   | –   | –   | 2   |
| South          | 21        | 39          | 13        | 9           | 10  | 1   | 1   | 1   | 8   | 1   | –   | –   |
| S1             | 14        | 31          | 10        | 7           | 8   | 1   | 1   | 7   | –   | –   | –   | –   |
| South          | 14        | 36          | 11        | 8           | 9   | 1   | 1   | 8   | –   | –   | –   | –   |
| E1             | 14        | 36          | 11        | 8           | 9   | 1   | 1   | 8   | –   | –   | –   | –   |
| East           | 16        | 29          | 13        | 11          | 10  | 1   | 1   | 9   | 2   | –   | –   | –   |
| W1             | 18        | 42          | 13        | 11          | 10  | 1   | 1   | 9   | 2   | –   | –   | –   |
| W2             | 22        | 38          | 5         | 3           | 2   | 1   | 2   | 3   | –   | –   | –   | –   |
| Total          | 134       | 271         | 80        | 58          | 59  | 8   | 8   | 5   | 51  | 4   | 1   | 2   |

*Sa* (*S. aureus*), *Se* (*S. epidermidis*), *Sh* (*S. hominis*), *Ss* (*S. simulans*).

strains out of the 110 isolates which coincided with their phenotypic characterization as MRSA isolates by the Kirby–Bauer method. When the DNA from *Staphylococcus* species other than *S. aureus* were examined by multiplex PCR, amplified PCR products were generated from the primers of the 16S rRNA gene only and not from those of the *nuc* gene. Two strains of *S. epidermidis* and one of *S. simulans* showed a positive PCR amplification of *mecA* gene hence detecting methicillin resistance in these two *Staphylococcus* species (Figure 5B).

4. Discussion

The human nasopharynx is occupied by a large number of bacteria some of which are commensal while others are considered to be potential pathogens [40].

Genus *Staphylococcus* comprises many unique species of which, *S. aureus* and other certain coagulase-negative staphylococci species have been implicated as main causal agents of community acquired as well as nosocomial infections. The coagulase-negative *S. epidermidis*, *S. hominis* and *S. simulans* species isolated in this study have been frequently reported to be involved in human infections, with *S. epidermidis* accounting for most infections [41–46].

So far only limited number of studies was conducted to highlight the nasal carriage of pathogenic *Staphylococcus* spp. among children in KSA. Instead, most of the research regarding staphylococcal species in KSA mainly focused only on infections caused by methicillin resistant *S. aureus* in the outpatient clinics of tertiary care hospitals for children [47–49]. Hence, we the authors believe that none of the previous studies...
discussed the asymptomatic distribution of *Staphylococcus* spp. nasal carriage among school children in KSA.

Colonization of the upper respiratory tract with potential *Staphylococcus* pathogens is very common in healthy children [50–52]. Our nasopharyngeal carriage study among the 405 elementary schools participants indicated that four blood hemolytic *Staphylococcus* spp. with carriage rate of 34.1% was detected among the participating school boys, while nasal cavities of the remaining participants (65.9%) did not bear any blood hemolytic bacterial species. When comparing this rate of carriage among the two age groups, we find the prevalence of nasopharyngeal colonization by each species to be affected by the student’s age. All four *Staphylococcus* spp. were slightly more prevalent (mean of 53%) in younger students attending schools and less prevalent (mean of 47%) in students of grades 4–6. Of the four species, *Staphylococcus aureus* was the most highly detected (79.7%) in both age groups of the elementary school boys. These carriage patterns coincide with most of the preceding studies showing the younger age group to have a higher carriage rate than the older ones in elementary schools [50–53]. Such findings propose that school children in this age group (7–9 years) have higher exposure risk most probably because of their less evolved adaptive immune response. This adaptive immunity is usually stimulated as a result of immunological memory over time, hence, providing an increased protection for young adult students of age groups 10–12 years [54].
Figure 5. Multiplex PCR assay to reveal methicillin resistance among isolated strains. (A) Multiplex PCR amplification profile. G1, *S. aureus* without mecA methicillin resistance; G2, *S. aureus* containing mecA methicillin resistance; G3, *Staphylococcus* spp. other than *S. aureus* without mecA methicillin resistance; G4, *Staphylococcus* spp. other than *S. aureus* with mecA methicillin resistance. (B) Frequency of bacterial isolates harbouring mecA gene. G1, *S. aureus* (64%) without mecA gene; G2, *S. aureus* (16%) containing mecA gene; G3, *S. epidermidis* (7.2%), *S. hominis* (6.5%), and *S. simulans* (4.3%) without mecA gene; G4, *S. epidermidis* (1.4%) and *S. simulans* (0.7%) with mecA gene. Lane M, DNA marker (100 bp ladder, Invitrogen, CA, USA).

It is fairly known that the results of epidemiological studies vary significantly from one study to another. The difficulty to compare the colonization rates of potential respiratory pathogens between different studies is related to the influence of numerous factors apart from the age, such as geographical area, climate, sampling technique, level of immunity, social and economic conditions [55,56]. Still, in contrast to many studies that focused on young children under 2 years of age, who frequently have high prevalence of *Staphylococcus* spp. carriage [47–49,56], this study paid more attention to two age groups that have been predisposed to the crowded urban life environment represented by elementary school boys in grade 1–3 (7–9 years) and older boys in grades 4–6 of school (10–12 years).

This study demonstrated that the colonization rate of *Staphylococcus* spp. varies according to the geographical location of the school. Furthermore, it was noticed that the lowest carriage number of *Staphylococcus* spp. was isolated from participants attending the two schools located in the west part of Al-Madinah city (18%) while higher carriage numbers were isolated from the remaining six schools (23–31%). The reason behind these differences may be related to the poor infrastructure of the schools as well as their overcrowding with students. Therefore, students in these schools are expected to have close contacts during most of the school activities which will subsequently lead to a higher risk of respiratory pathogens transmission among them. Another explanation may be related to the standards of living and socioeconomic status since school boys of the west part of Al-Madinah are more likely to be from higher social as well as economic status which may reflect on their proper hygiene practices. One drawback of this study was the low number of participating students (only 25 participants) from one of the schools located in the northern area of Al-Madinah city. This occurred due to the fact that some of the participating students’ parent or legal guardians hesitated after signing the consent forms, and since the authors made sure to inform the students as well as parent or legal guardians that they can stop or withdraw at any time, the results regarding the previous group of participants had to be excluded from the study. Still, the samples that were successfully collected from 60 participants in a second school that shares the same geographical location (reaching a total of 85 participants) has reassured the authors that an accurate investigation of the carriage was properly undertaken.

Collectively, the epidemiological data from this study supports that there is a significant increase in *S. aureus* carriage among the participating students. Such result has also been acknowledged in previous studied since *S. aureus* is well known to primarily reside in the anterior nares. Therefore, *S. aureus* carriage is more frequently detected (36–65%) in nasal, oral or nasopharyngeal swabs [57]. On the other hand, the different carriage rate among coagulase-negative staphylococci in this study may involve a multifarious combination of factors that influence the participants exposure to a specific bacterial species, most important are participants immune responses and receptor-binding sites as well as direct competitive interactions between coagulase-negative staphylococci isolated in this study and other bacterial species in the same local niche [58].

Since *S. aureus* was the most frequently isolated staphylococcal bacteria in this study, a simple and rapid technique for identifying as well as discriminating of *S. aureus* from other coagulase-negative staphylococci was accomplished through conventional multiplex PCR. Amplifying the 16S rRNA and nuclease (*nuc*) encoding genes allowed the accurate identification of *S. aureus* in
a single step. The results of the conventional multiplex PCR performed in this study, confirmed the specificity of the 16S rRNA gene primer pair in amplifying all Staphylococcus species and the nuc primers specificity for S. aureus isolates as reported previously [21,23,34,39].

The detection of S. aureus isolates showing resistance towards methicillin is essential especially when it involves the frequently targeted subjects (school age children) by these bacteria as reported in this study as well as several other studies [50–52]. The importance of such screening for MRSA will be reflected upon both, proper patient care through a sufficient supply of effective antibacterial drugs and infection control by restraining the use ineffective classes of antibiotics. The examination of MRSA can be done either phenotypically by Kirby–Bauer disc diffusion method or genotypically through detecting the presence of mecA gene which normally indicates a possible resistance to beta-lactam antibiotics.

In this study, it was found that all 20% (22/110) of the S. aureus isolates that were identified as methicillin resistant by the Kirby–Bauer method showed the presence of mecA gene. Such data indicates a close association between resistance towards methicillin and the presence of mecA gene in the tested isolates. Other studies on S. aureus isolates showed similar results in which most of the investigated isolates of MRSA certainly had mecA gene [59–61]. Such resistance towards methicillin along with all beta-lactam groups of antibiotics is related to a mutation that resulted in changing the normal antibiotic binding protein which resulted in lowering the binding affinity of beta-lactams to their site [62]. Additionally, during the same study mecA gene amplification was identified in two S. epidermidis isolates and one S. simulans isolate. The detection of mecA gene in Staphylococcus species apart from S. aureus was previously confirmed [63,64].

5. Conclusion

In view of the findings presented in this study, asymptomatic nasopharyngeal colonization by blood haemolytic staphylococcal especially S. aureus among elementary school boys was found to be high. Unfortunately, those asymptomatic carriers are considered to be a potential threat to other healthy students, emphasizing the need to develop crucial approaches in order to reduce the carriage of such potential respiratory pathogen. Hence, constant screening for carriers is necessary to minimize the transmission of respiratory infections as well as the occurrence of bacterial pathogens among school students.

The examination of the antimicrobial susceptibility pattern among the isolated blood haemolytic staphylococcal in this study revealed an epidemiological shift in S. aureus isolates with 20% of them showing mecA gene occurrence accompanied by resistance towards methicillin which prompts the need for sufficient control measures to avoid the spread of these MRSA isolates. The multiplex-PCR based identification technique used in our study has proven to be both quick and precise for the differentiation between coagulase-positive and negative staphylococci as well as the detection of MRSA isolates that might be circulating in the schools.

Based on the previous data, surveillance programs must be carried out in different parts of KSA for a better understanding of the URT colonization rate by the targeted bacteria and the frequency of successive respiratory infection. This may be important for future prediction of any changes in the epidemiology of URT infectious staphylococci and will help implementing accurate guidelines for control strategies of potential pathogenic bacteria colonizing the URT.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Author contributions

Each one of the authors has made an equal contribution in conceptualizing and implementing the methodology, analysing the data as well as writing and revising the manuscript.

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