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Determination of antibiotic resistance genes, immune evasion cluster and *agr* types among *Staphylococcus aureus* strains isolated from children with adenoiditis

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ABSTRACT

Adenoiditis is the most common infection in childhood and different microorganism such as viruses and bacteria cause this infection. *Staphylococcus aureus* (*S. aureus*) is the main Gram-positive bacterium that can involve in adenoiditis. The aim of this study was to determination of antibiotic resistance, immune evasion cluster genes and *agr* types among *S. aureus* isolates were collected from children with adenoiditis. Totally 36 clinical isolates of *S. aureus* were obtained from 112 children suffering from adenoiditis. Susceptibility of the isolates to different antimicrobial agents were determined using standard disk diffusion method. PCR technique was used for detection of resistance and virulence genes. SCC*mec* and *agr* typing were used for molecular typing of the isolates. All isolates were sensitive to vancomycin and 47.2% of isolates were resistant to penicillin. Twenty-two present of isolates were considered as MRSA and SCC*mec* type IV was detected in 5 MRSA isolates. Only, *agr* types I and II were identified among the isolates. *ermC* was the predominant macrolide resistance gene and *ant(4′)-Ia* was the most common aminoglycoside resistance gene. Virulence genes including *sak*, *chp* and *sea* were identified in 47.2% (n = 17), 30.5% (n = 11) and 13.8% (n = 5). This study was the first report in Iran, Kerman, about determination of antibiotic resistance, virulence genes, *agr* groups and SCC*mec* types among the clinical *S. aureus* isolates were collected from children with adenoiditis. Also, our results can be helpful in empirical therapy and increase our knowledge about genetic characteristics of *S. aureus* isolates are involved to adenoiditis.

1. Introduction

The inflammation and enlarged of the adenoid tissue called adenoiditis. Adenoiditis is one of the most important infections in childhood (Rajeshwary et al., 2013). Adenoiditis usually occurred by viruses, however some other microorganisms such as bacteria including *Staphylococcus aureus* (*S. aureus*), *Moraxella catarrhalis*, group A beta-hemolytic *Streptococcus*, *and Haemophilus influenzae* colonize on the

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; SCC*mec*, Staphylococcal Cassette Chromosome *mec*; IEC, immune evasion cluster; agr, accessory gene regulatory; PCR, polymerase chain reaction; CLSI, Clinical and Laboratory Standards Institute; CA, community-associated; HA, hospital-acquired

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adenoid tissue and can cause the adenoiditis (Rajeshwary et al., 2013; Emaneini et al., 2015). The adenoiditis usually treats using different antibiotics, but, if the adenoiditis severe or recur may require removal of the adenoids by surgical procedures (Nistico et al., 2011).

S. aureus is the most common Gram-positive bacterium is involved in adenoiditis (Lin et al., 2012; Rajeshwary et al., 2013). In the several studies were shown that *S. aureus* can isolates from the adenoid tissue of children with adenoiditis or adenoid hypertrophy (Emaneini et al., 2015; Lin et al., 2012; Rajeshwary et al., 2013). Spread of antibiotic resistance can lead to the antibiotic therapy failure in adenoiditis (Emaneini et al., 2015). In recent years, the resistance of *S. aureus* to various antibiotics such as methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are increasing (Lin et al., 2012; Rajeshwary et al., 2013). MRSA isolates are usually resistant to several classes of antibiotics such as aminoglycosides, erythromycin and clindamycin, so treatment of infections caused by them are difficult (Javdan et al., 2019). Staphylococcal Cassette Chromosome *mec* (SCC*mec*) which is located on mobile genetic elements is responsible to methicillin resistance in MRSA isolates by encodes modified penicillin-binding proteins called PBP2a (Palavecino, 2014). *msrA/B* gene is a ATP-binding cassette (ABC) transporters encoding efflux pump, that can cause resistance to macrolides also *ermA/B/C* by methylation of ribosome subunit, are the main resistance genes to macrolides antibiotics such as erythromycin and clindamycin. The aminoglycosides modification enzymes (AMEs) encoding genes including (*aac(6′)-Ie-aph(2″)-I*, *aph(2″)-Ib*, *aph(2″)-Ic*, *aph(2″)-Id*, *aph(3′)-IIIa*, *and ant(4′)-Ia*) have an important role in increasing of resistance to these antibiotics (Goudarzi et al., 2020).

The *Staphylococcus aureus* immune evasion cluster (IEC) is the innate immune modulators and located on β-haemolysin-converting bacteriophages (βC-Φs) and encoded various virulence genes including *scn*, *sea*, *sak*, *sep* and *chp*, these cluster protect of *S. aureus* from immune defense systems (van Wamel et al., 2006). The accessory gene regulatory (*agr*) operon including *agrA*, *agrB*, *agrC*, and *agrD* genes is one of the major regulatory system for expressing and controlling virulence genes and pathogenicity in *S. aureus*. Some studies show relation between type of *agr* with presence of virulence genes and antibiotic resistance (Javdan et al., 2019). Since, understanding the mechanisms of drug resistance and virulence factors as well as the genetic relatedness of bacteria is important in treatment and prevention strategies, in this study we were determined profile of antibiotic resistance, resistance and virulence genes and molecular relatedness among clinical isolates *S. aureus* from adenoiditis in Kerman, Iran.

2. Material and methods

2.1. Bacterial isolates

During 2018 to 2019, totally 36 bacterial isolates of *S. aureus* were obtained from adenoid tissue 112 patients with symptoms of adenoid hypertrophy with age range 2 to 5 years. These patients were admitted at the department of otolaryngology at Shafa Teaching Hospitals, Kerman, Iran and were undergone for adenoidectomy. All isolates identified as *S. aureus* by standard microbiological tests and amplification of the *nuc* gene by polymerase chain reaction (PCR) technique was used for molecular confirmation of the isolates (Goudarzi et al., 2020). The list of primers was used for detection of *nuc*, resistance genes, IEC genes, and *agr* typing were shown in Table 1.

2.2. Antibacterial susceptibility tests

The susceptibility of isolates to different antibiotics agents including penicillin (P, 10 unit), gentamicin (GM, 10 μg), amikacin (AM, 30 μg), tobramycin (TOB, 10 μg), erythromycin (E, 15 μg), ciprofloxacin (CIP, 5 μg), levofloxacin (LEV, 5 μg), norfloxacin (NOR, 10 μg), tetracycline (TE, 30 μg), clindamycin (CL, 2 μg) and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg), were determined by disk diffusion method (CLSI, 2020). In this study we use cefoxitin (FOX, 30 μg) disk and D-zone test for detection of MRSA and inducible clindamycin resistance isolates, respectively (CLSI, 2020).

2.3. Total DNA extraction

Total DNA of the isolates was extracted by boiling method previously described by Montazeri et al. (2015).

2.4. Detection of antibiotic resistance genes and immune evasion cluster (IEC) genes

Polymerase chain reaction (PCR) was used for detection of *ermA*, *ermB*, *ermC* and *msrA/B* and presence of immune evasion cluster (IEC) genes including *scn*, *sea*, *sak*, *sep*, *chp* (Goudarzi et al., 2020; van Wamel et al., 2006). Multiplex PCR was used for amplification aminoglycoside modifying enzymes (AMEs) genes; (*aac(6′)-Ie-aph(2″)-I*, *aph(2″)-Ib*, *aph(2″)-Ic*, *aph(2″)-Id*, *aph(3′)-IIIa*, *and ant(4′)-Ia*) (Goudarzi et al., 2020). The list of primers was used for detection of *nuc*, resistance genes, IEC genes, and *agr* typing were shown in Table 1.

2.5. agr typing of the isolates by multiplex-PCR

Four groups of *agr* types (I-IV) were detected by multiplex PCR method (Shopsin et al., 2003). The nucleotide sequences of the *agr* typing primers *agr*-IR (*agr* group I), *agr*-II (*agr* group II), *agr*-IIIR (*agr* group III), *agr*-IV (*agr* group IV), and *agr*-pan-F were listed in Table 1. The multiplex PCR were performed using Taq DNA Polymerase 2× Master Mix RED (Ampliqon, Co, Denmark) according to manufacturer's recommendation. The PCR amplicons were stained by Green Viewer safe dye (Sinaclon, Co, Iran) and visualized using Gel Documentation UV light System after electrophoresis in a 1.5% agarose with 0.5× TBE (Tris/Borate/EDTA) buffer.

2.6. SCCmec typing of MRSA isolates

SCC*mec* types of the MRSA isolates were determined by multiplex-PCR method using four primer-pairs for discrimination of SCC*mec* types I-V previously described by Boye et al. (2007). The multiplex PCR were performed using Taq DNA Polymerase 2× Master Mix RED (Ampliqon, Co, Denmark) according to manufacturer's recommendation. The PCR products were stained by Green Viewer safe dye and visualized using Gel Documentation UV light System after electrophoresis in a 1.5% agarose with 0.5× TBE (Tris/Borate/EDTA) buffer. The SCC*mec* types were determined on the basis of the size of the PCR amplicons.

2.7. Statistical analysis

The data was statistically analyzed using SPSS version 23 (IBM, Armonk, NY, USA). The chi square test and the Fisher exact test, applied to compare categorical variables and *P*-values ≤0.05, were considered to be statistically significant.

3. Results

In this study, totally 36 (31.14%) *S. aureus* isolates were collected from the adenoid tissues of the 112 patients with adenoid hypertrophy. The antimicrobial susceptibility testing results showed 97/2% (n = 35), 69.4% (n = 25), 47.2% (n = 17) and 47.2% (n = 17) of the isolates were resistance to penicillin, tetracycline, erythromycin and tobramycin, respectively. All of the isolates were sensitive to vancomycin and gentamicin. The resistance to the other antibiotic agents was; 5.5% (n = 2) to amikacin, 11.1% (n = 4) to ciprofloxacin, 2.7% (n = 1) to levofloxacin, 5.5% (n = 2) to norfloxacin, 5.5% (n = 2) to clindamycin, 25% (n = 9) to trimethoprim/sulfamethoxazole. Twenty-two

Table 1
The list of primers and annealing temperature (°C) for genes investigated in this study.

Gene target	Primer sequence (5′-3′)	Annealing temperature (°C)	Product (bp)
nuc	F-GCGATTGATGGTGATACGGTT	60	279
	R-AGCCAAGCCTTGACGAACTAAAGC		
mecA	F-TCCAGATTACAACTTCACCAG G	56	162
	R-CCACTTCATATCTTGTAACG		
erm A	F-TATCTTATCGTTGAGAAGGGATT	56.5	139
	R-CTACACTTGGCTTAGGATGAAA		
erm B	F-CTATCTGATTGTTGAAGAAGGATT	56.5	142
	R-GTTTACTCTTGGTTTAGGATGAAA		
erm C	F-AATCGTCAATTCCTGCATGT	55.5	297
	R-TAATCGTGGAATACGGGTTTG		
msrA/B	F-GCAAATGGTGTAGGTAAGACAACT	56.5	402
	R-ATCATCATGTGATGTAAACAAAAT		
aac(6′)-Ie-aph(2″)-I	F-CAGGAATTTATCGAAAATGGTAGAAAAG	60	369
	R- CACAATCGACTAAAGAGTACCAATC		
aph(2″)-Ib	F-CTTGGACGCTGAGATATATGAGCAC	60	867
	R-GTTTGTAGCAATTCAGAAACACCCTT		
aph(2″)-Ic	F-CCACAATGATAATGACTCAGTTCCC	60	444
	R-CCACAGCTTCCGATAGCAAGAG		
aph(2″)-Id	F-GTGGTTTTTACAGGAATGCCATC	60	641
	R-CCCTCTTCATACCAATCCATATAACC		
aph(3′)-IIIa	F-GGCTAAAATGAGAATATCACCGG	60	523
	R-CTTTAAAAAATCATACAGCTCGCG		
ant(4′)-Ia	F-CAAACTGCTAAATCGGTAGAAGCC	60	294
	R-GGAAAGTTGACCAGACATTACGAACT		
chp	F-GAAAAAGAAATTAGCAACAACAG	48	410
	R-CATAAGATGATTTAGACTCTCC		
sak	F-AAGGCGATGACGCGAGTTAT	50	223
	R-GCGCTTGGATCTAATTCAAC		
sea	F-AGATCATTCGTGGTATAACG	50	408
	R-TTAACCGAAGGTTCTGTAGA		
sep	F-AATCATAACCAACCGAATCA	50	500
	R-TCATAATGGAAGTGCTATAA		
scn	F-AGCACAAGCTTGCCAACATCG	49	258
	R-TTAATATTTACTTTTTAGTGC		
agr	Pan-ATG CAC ATG GTG CAC ATG C	50	–
	I-GTCACAAGTACTATAAGCTGCGAT		441
	II-TATTACTAATTGAAA AGT GGC CATAGC		575
	III-GTA ATG TAATAGCTTGTATAATAATACCCAG		323
	IV-CGATAATGCCGTAATACCCG		659

percent (n = 8) of the isolates were MRSA and 41.6% (n = 15) of them were considered as inducible clindamycin resistance according D-zone test results.

erm A, *B* and *C* genes were detected in 5.5% (n = 2), 11.1% (n = 4) and 25% (n = 9) of the isolates, respectively. IEC genes including *sak*, *chp* and *sea* were identified in 47.2% (n = 17), 30.5% (n = 11) and 13.8% (n = 5) of the isolates. *msrA/B*, *sep* and *scn* genes were not detected in this study.

The aminoglycoside resistance genes *ant(4′)-Ia* and *aac(6′)-Ie-aph (2″)-I* were detected in 41.6% (n = 15) and 5.5% (n = 2) of the isolates.

Among MRSA (n = 8) isolates, *mecA* gene were observed in 62.5% (n = 5) of them and all of *mecA* positive isolates were belonging to SCC*mec* type IV. *agr* type I was the predominant *agr* types among the isolates with prevalence 38.8% (n = 14), that followed by *agr* type II with prevalence 25% (n = 9). *agr* types III and IV were not observed among the isolates. A significant coloration was observed among the *agr* types I and II with presence of *erm*, AMEs and virulence genes (*P*-value ≤ 0.05). Also a significant relation was observed to presence of *ant (4′)-Ia and aac(6′)-Ie-aph(2″)-I* genes and resistance to tobramycin (P-value ≤ 0.05). Distribution of antibiotic resistance profile, virulence and resistance genes, SCC*mec* and *agr* types among 36 clinical isolates of *S. aureus* were shown in Table 2.

4. Discussion

The adenoids can play an important role in infectious and non-infectious upper airway disorders in children. Usually, adenoidectomy and different antibiotics agents use for controlling and treatment of adenoiditis. Having data about the profile of antibiotic resistance among bacterial that causes the adenoiditis plays an important role in proper antibiotic treatment. On the other hand, knowing the virulence factors of the pathogens can also give us a prognosis for tissue damage.

In this study 32.14% of the children's adenoid tissue was colonized with *S. aureus*. In a study in Tehran, Iran by Emaneini et al. (2011), *S. aureus* isolates were collected from 23% of the children's adenoid tissue. In another study by Lin et al., in Taiwan, the prevalence of *S. aureus* in the children's adenoid tissue was reported 21.2% (Lin et al., 2012). These results were show important role of *S. aureus* in the adenoiditis or adenoid hypertrophy in childhood.

The antibiotic resistance and prevalence of MRSA strains are increasing among different clinical isolates of *S. aureus* in both community-associated (CA) and hospital-acquired (HA) infections (Moosavian et al., 2020). Antibiotic resistance and presence of virulence factors or regulatory system genes can explain the persistence, stability and colonization of bacterial pathogenic in the nasopharynx and upper airway tract (Lin et al., 2012; Rajeshwary et al., 2013).

Data about clinical isolates of *S. aureus* were isolated from adenoiditis is rare in Iran. In this study, we reported SCC*mec*, *agr* types, ICE cluster and some resistance genes for the first time among the *S. aureus* isolates were collected adenoiditis in Kerman, Iran.

In present study, similar to other studies, resistance to penicillin was more than other antibiotic agents among *S. aureus* isolates and all isolates were sensitive to vancomycin (Lin et al., 2012; Rajeshwary et al., 2013). Also, we observed low rate of resistance to amikacin, ciprofloxacin, levofloxacin, norfloxacin, and clindamycin among the isolates.

Table 2
Distribution of antibiotic resistance profile, MRSA isolates, virulence and resistance genes, SCC*mec* and *agr* types among *S. aureus* isolates.

Isolates	Antibiotic resistance profile	MRSA	mecA	SCC*mec*	agr	Resistance genes	Viruence genes	iCR
1	P	−	−	−	I	−	sak, chp	−
2	SXT, P, TE, E	−	−	−	I	ermC	sak	+
3	SXT, P, CIP, TE, NOR, E, CL, LEV	−	−	−	II	ermB	sea, sak	−
4	SXT, P, E	−	−	−	II	ermC	chp	+
5	SXT, P	−	−	−	II	−	sak	−
6	P	−	−	−	I	−	sak, chp	−
7	P	−	−	−	I	ermB	sak, chp	−
8	TOB, P, CIP, TE, E	−	−	−	I	ant(4′)-Ia	sak, chp	−
9	TOB, P, TE	−	−	−	−	ant(4′)-Ia	sak, chp	−
10	P, FOX, TE, E	+	+	IV	−	ermC	sak	+
11	TOB, P, TE, E	−	−	−	I	ant(4′)-Ia	sak	−
12	SXT, TOB, P, FOX, TE, E	+	−	−	I	ermB, ant(4′)-Ia,	sak, chp	+
13	TOB, P, FOX, TE, E	+	+	IV	I	ermC, ant(4′)-Ia	sea, sak	+
14	P, FOX, TE, E	+	−	−	I	−	sea, sak	−
15	SXT, TOB, P, TE, E	−	−	−	−	ermA, aac(6′)-Ie-aph(2″)-I	sak, chp	−
16	TOB, P, CP, TE, NOR, E, CL, AM	−	−	−	II	ermB, ermC, aac(6′)-Ie-aph(2″)-I	chp	−
17	TOB, P, TE, R	−	−	−	−	ant(4′)-Ia	−	+
18	TOB, P, TE, E	−	−	−	II	ant(4′)-Ia	−	+
19	TOB, P, CIP, TE, E	−	−	−	II	ant(4′)-Ia	−	−
20	P	−	−	−	I	−	−	+
21	TOB, P, FOX, TE, E	+	+	IV	I	ant(4′)-Ia	−	−
22	TOB, P, TE	−	−	−	−	ant(4′)-Ia	sea	−
23	TOB, P, TE	−	−	−	II	ermA, ant(4′)-Ia	−	−
24	SXT, E	−	−	−	I	ermC	−	+
27	TOB, P, TE, E	−	−	−	−	ermC, ant(4′)-Ia	−	+
28	SXT, P, TE, E	−	−	−	II	ermC	−	+
29	P, FOX, TE	+	+	IV	−	−	sak	−
30	P, E	−	−	−	−	ermC	sak, sea	+
31	P,	−	−	−	−	−	sak	−
32	SXT, P, TE	−	−	−	II	−	chp	−
33	TOB, P, TE, E	−	−	−	II	ant(4′)-Ia	−	+
34	TOB, P, TE	−	−	−	II	ant(4′)-Ia	−	−
35	P, FOX, TE, E	+	−	−	−	−	−	+
36	P, FOX	+	+	IV	−	−	−	−
37	TOB, P, TE, AM	−	−	−	−	ant(4′)-Ia	−	−

MRSA: methicillin-resistant *Staphylococcus aureus*, iCR: inducible clindamycin resistance, P: penicillin, SXT: trimethoprim/sulfamethoxazole, TE: tetracycline E: erythromycin, CIP: ciprofloxacin, NOR: norfloxacin, CL: clindamycin, LEV: levofloxacin, TOB: tobramycin, FOX: cefoxitin, AM: amikacin.

However, resistance to other antibiotics including tetracycline, erythromycin, tobramycin, and trimethoprim/sulfamethoxazole is considerable for use of them in treatment of adenoiditis.

Among the isolates 22.2% ($n = 8$) of them were MRSA. In study by Lin et al., the prevalence of MRSA isolates in patients with adenoiditis were 35% in Taiwan (Lin et al., 2012). None significant correlation was observed among antibiotic resistance genes, *agr* and SCC*mec* types in MRSA isolates. SCC*mec* type IV was detected among *mecA* positive isolates, this finding was accordance with lineage of SCC*mec* IV in community-associated infection by MRSA (CA-MRSA), because according to reports SCC*mec* IV is the predominant SCC*mec* type among CA-MRSA (Ito et al., 2014).

Our results showed significant correlation in presence of *erm* and aminoglycoside modification genes (*aac(6′)-Ie-aph(2″)-I* and *ant(4′)-Ia*) with resistance to erythromycin and tobramycin, respectively (Table 2).

The rate of *erm* genes in inducible clindamycin (iCR) resistance isolates was higher than iCR negative isolates. The *erm* genes by methylation of large ribosome subunit have an important role in increasing resistance to macrolide antibiotics and can cause antibiotic therapy failure by erythromycin and other macrolide antibiotics (Fasihi et al., 2017). In 2017, a study by Fasihi et al. in Kerman, Iran on various clinical samples of *S. aureus*, *ermA* and *ermC* genes have been reported in 4 and 16% of the isolates (Fasihi et al., 2017). In our study the rate of *ermA*, *ermB* and *ermC* were reported 5.5% ($n = 2$), 11.1% ($n = 4$) and 25% ($n = 9$) of the isolates. These results reflect the increase of macrolide resistance genes in community-associated infection isolates of *S. aureus*. Also, in this study, the rate of resistance to tobramycin was high and all of tobramycin resistant isolates had *ant(4′)-Ia* and *aac(6′)-Ie-aph (2″)-I* genes. So, we suggest that more attention should be paid to the use of antibiotic in the treatment of infections caused by community-associated bacteria.

The *agr* type I was the most common *agr* type among the isolates. This finding similar to other studies have been reported the *agr* type I is the most predominant *agr* type in clinical isolates of *S. aureus* (Javdan et al., 2019; Silva et al., 2020). Also, in some reports were declared that *agr* is involved with invasive infection and antibiotic resistance genes. In our study, two *agr* type I and II were detected and significant correlation was observed among the *agr* types I and II with presence of *erm*, aminoglycoside resistance genes and virulence genes. Also, the prevalence of resistance and virulence genes were very low among *agr* negative isolates. These result and other reports support the relation between presence of *agr* with pathogenicity and antibiotic resistance in *S. aureus*.

5. Conclusion

This research is the first report from Iran, about antibiotic resistance and virulence genes, *agr* gruop, SCC*mec* type among clinical isolates of *S. aureus* collected from adenoiditis. The importance outcome of our investigation was to provide evidence that *S. aureus* has an important role and relation with adenoiditis or adenoid hyperplasia in children. This study shows high prevalence of resistance genes among *S. aureus* from adenoiditis that can cause failed in antibiotic therapy and can increase our knowledge about genetic characterization of *S. aureus* strains are involved to adenoiditis.

CRediT authorship contribution statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by: [Mahsa Ziasistani, Shahriar Dabiri, Maryam Fekri Soofi, Setareh Agha Kuchak Afshari, Rasoul Ghaioumy, Fatemehalsadat Tabatabaeifar, Davood Kalantar-Neyestanaki]. The first draft of the manuscript was written by [Davood Kalantar-Neyestanaki and José Rubén Morones Ramírez] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

None declared.

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