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PCR-mediated identification of Methicillin and Vancomycin resistant genes in *Staphylococcus aureus* strains isolated from the nasal cavity

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ABSTRACT

Staphylococcus aureus is colonized in the human nasal cavity and would contaminate hospital and therapeutic environments. This bacterium has a genetic diversity in terms of resistance to antimicrobial agents. Therefore, the aim of this study was identification of Methicillin and Vancomycin resistant genes in *Staphylococcus aureus* strains which has been isolated from the nasal cavity. 189 patients referred to health centers of Amol County, Iran were sampled. Resistance to antibiotics of Vancomycin, Methicillin, Cefepime, Ceftriaxone, Cefixime, Nalidixic acid, Cefazolin, Cefotaxime, Ceftazidime, Imipenem and Ciprofloxacin were identified by disk diffusion method and recommendations of the Clinical and Laboratory Standards Institute (CLSI). Polymerase chain reaction (PCR) was performed to detection of *mecA*, *vanA* and *femB* genes. 32.2% of the patients were carriers of *Staphylococcus aureus* in their nose. In this research, 68.85% of the isolates were resistant to Methicillin. A hundred percent resistance to Cefixime and Nalidixic Acid were observed. Subsequently, the highest rates of resistance belonged to Cefazolin (96.72%) and Cefepime (86.88%). Intermediate Resistance to Vancomycin was 4.91%. The lowest rates of resistance were observed toward two antibiotics Ciprofloxacin and Imipenem, with rates of 6.55% and 9.83% in isolates, respectively. Genetically, no resistance was observed for Vancomycin by PCR technique. Of 61 isolates of *Staphylococcus aureus*, 77.04% of and 90.16% were carried the *mecA* gene and *femB* gene respectively. This study showed that the main resistance to Methicillin in Amol County is due to *mecA* and *femB* genes.

1. Introduction

What has attracted the attention of health centers and physicians in recent years is the resistance of bacteria such as *Staphylococcus*, *Enterococcus*, *Klebsiella* and *Pseudomonas* to antibiotics. However, resistance to antibiotics in these bacteria has led to the ineffectiveness of treatment and adverse consequences (Tenover, 2006). Methicillin-resistant Staphylococci and vancomycin-resistant Enterococci are among the most important causes of nosocomial infections (Malani et al., 2002). Staphylococci are gram-positive coccoi that produce Catalase enzyme (Juyal et al., 2013). *Staphylococcus aureus* is the most aggressive species, and in developing countries it is one of the most important agents in bacterial infections, which are caused by

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simple skin infections such as boils, abscesses, carbuncle and also life-threatening diseases such as pneumonia, Meningitis, Osteomyelitis, endocarditis, toxic shock syndrome and septicemia (Turlej et al., 2011). This bacterium produces yellow colonies due to the production of a golden colored carotenoid called Staphyloxathin. This pigment plays a role in pathogenicity, as it acts as an antioxidant agent, and helps to keep the bacteria safe against oxygen free radicals (Clauditzet al., 2006). Free oxygen radicals are produced by the immune system (white blood cells) in hosts to kill bacteria. *Staphylococcus aureus* also plays a role in food poisoning, which occurs through the production of enterotoxin (Salyers and Whitt, 2002). These bacteria have the ability to survive on dry surfaces from several weeks to several months (Gould and Chamberlaine, 1995). Since penicillin was introduced for clinical purposes, resistance to Beta-Lactam antibiotics has increased. This type of resistance was due to the presence of penicillinase encoding plasmid. Penicillinase deactivates the molecule of antibiotics by breaking the beta-lactam ring (Deurenberg et al., 2007). Nasal carriers are among the main causes of staphylococcal infections (Creechet al., 2005). The anterior portion of the nose has been the primary source of *Staphylococcus aureus* in adults and children and from 20 to 40 percent of the society's healthy people are nasopharyngeal (the nasal carriers of *Staphylococcus aureus*) (Ramanaet al., 2009). Resistance to Methacillin is created by producing a specific penicillin-binding protein called PBP2a. This protein has a very weak affinity for Beta-Lactam antibiotics. PBP2a is coded by the *mecA* gene and carried by the large cassette of the SCCmec3 gene that is mobile. So far, seven types of SCCmec (I-VII) have been identified and still are increasing (Otter and French, 2010). In 2002, the first clinical sample of Vancomycin resistant *Staphylococcus aureus* was isolated from the patient at state of Michigan, USA (Chang et al., 2003). Vancomycin-resistant *Staphylococcus aureus* is likely to be resistant to Vancomycin via the VanA gene (Noble et al., 1992). Resistance factors are often found in mobile genetic elements, such as transposons or conjugate plasmids, which facilitate transportation of resistance genes through the horizontal transfer of genes to other bacteria (Facklam and Collins, 1989). Since the identification of Vancomycin and Methycillin resistant species and studying the release of these species is important for epidemiological purposes and control of infections caused by these bacteria, so this research has been developed to isolate and identify Methicillin and Vancomycin resistant genes in *Staphylococcus aureus.*

2. Materials and Methods

2.1. Sample collection and initial identification

The present study was done on 189 patients referred to the health centers during the February-2016 and January- 2017. Sampling was carried out with a wet swab from the nasal cavity. To separate *Staphylococcus aureus*, the swabs were immediately cultured in mannitol salt agar culture media. *Staphylococcus aureus* strains were identified using conventional methods such as gram stain, catalase, coagulase, mannitol sugar fermentation, sensitivity to Novobiocin and DNase (Cui et al., 2003).

2.2. Investigating the Antibiotic susceptibility by phenotypic method

Minimum inhibitory concentration was used to evaluate the resistance of the strains to Methicillin and Vancomycin antibiotics. Resistance to Methicillin was evaluated by Broth micro-dilution method. For this purpose, different dilutions of Methicillin with a specific concentration of *Staphylococcus aureus* suspension with 0.5 MacFarland Turbidity were mixed in the micro- plates. The specimens were subsequently incubated for 24 hours. By determining the opacity of the wells, the minimum inhibitory concentration of antibiotic was determined (Rahimi-Alang et al., 2011). Evaluation of sensitivity of *Staphylococcus aureus* to Vancomycin was performed due to CLSI guidelines in the way that dilution agar method was used. In this method, a specific concentration of *Staphylococcus aureus* suspension with 0.5 MacFarland Turbidity was inoculated into the Mueller-Hinton agar medium containing Vancomycin. The specimens were incubated for 24 hours and then the lowest inhibitory concentration of bacteria was determined. The disk diffusion method was also used to investigate the sensitivity of other

antibiotics such as Cefeipime, Ceftriaxone, Cefazolin, Ceftizoxime, Cefotaxime, Ceftazidime, Imipenem, Nalidixic Acid and Ciprofloxacin (Clinical, Institute, & Patel, 2015). An antibiotic test with 0.5 MacFarland Turbidity was performed from *Staphylococcus aureus* suspension in a Mueller Hinton agar culture medium. For this purpose, samples were incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured in millimeters and the result was recorded as resistant, intermediate, and sensitive. To control the quality of the test, the standard strain of *Staphylococcus aureus* was used as a positive control.

2.3. Testing of antibiotic susceptibility by genotyping method

DNA extraction and amplification of *vanA*, *femB* and *mecA* genes from the specimens were performed in the genetic lab of the Pasteur Lab Institute of Iran. To extract DNA, we use Fermentase kit and in accordance with the manufacturer's instructions. The *vanA*, *femB* and *mecA* genes were amplified using specific primers (Table 1) and PCR method.

Table 1. Information of Specific Primers (5'-3 ') used in the present research of primers used for PCR

gene	Primer	Primer sequence (5'-3 ')	Primer length	temperature of Tm	GC percent	**amplicon length (bp)**
vanA	Forward	GTATTGGGAAACAGTGCCGC	20	59.83	55	316
	Reverse	CGGCCATCATACGGGGATAA	20	59.39	55	
mecA	Forward	GTAGAAATGACTGAACGTCCGATAA	25	58.97	40	344
	Reverse	ACGATGCCTATCTCATATGCTGT	23	59.49	50	
emB	Forward	TCGCATGGTTACGAGCATCA	20	59./83	50	521
	Reverse	AGGTTTAGAATCGGGCCGTC	20	59.82	55	

The final volume of the main mixture was considered to be 20 µL, which included 2 µL of DNA template, dNTP with a concentration of 0.4 µL for each of the nucleotides, 0.6 µL MgCl2, 2 µL of 10x PCR buffer, 0.2 µL of the Taq DNA Polymerase enzyme (fermentase) and 0.5 µL of each primer (20 pmol) From each of the primers 12.6 µl of distilled water The Prime-Mid-Size Thermal Cycler model belonging to Techno company's Thermo Cyclic Gradient System was used. The thermal program used for the *vanA* gene was as follows: a 95°C cycle for 4 minutes, 32 repetitive cycles of 95°C for 45 seconds, 58°C for 1 minute, 72°C for 45 seconds, the final cycle was 72°C for 6 minutes. The thermal program used for the *mecA* gene was as follows: a 95°C cycle for 5 minutes, 34 cycles of 95°C for 50 seconds, 57°C for 45 seconds, 72°C for 1 minute and the final cycle was 72°C for 7 minutes. Also, *femB* gene was as follows: a 95°C cycle for 4 minutes, 30 cycles of 95°C for 1 minute, 56°C for 1 minute, 72°C for 1 Minutes and the final cycle was 72°C for 6 minutes. PCR products were electrophoresed in 2% agarose gel containing Ethidium bromide. The DNA fragments were observed using UV transilluminator device. Data were analyzed by SPSS software version 16.

3. Results

Of the 189 samples taken from the Nasal cavity through swab, 61 strains of *Staphylococcus aureus* were approved. In this study, 32.2% of the persons had *Staphylococcus aureus* in their nose. The percentage of susceptible, resistant, and intermediate susceptibility to antibiotics has been shown in the Table 2.

Table 2. Sensitivity Frequency (percentage) of isolated *Staphylococcus aureus* to different antibiotics

Family	Antibiotics	Resistant Number (%) of isolates	Intermediate Number (%) of isolates	Sensitive Number (%) of isolates
Penicillins	Methicillin	42 (68.85)	18 (29.50)	1 (1.63)
Cephalosporines	Cefazolin	59 (96.73)	0	2 (3. 27)
	Cefepime	53 (86.88)	6 (9.83)	2 (3.27)
	Cefotaxime	32 (52.45)	29 (47.54)	0
	Ceftazidime	10 (16.39)	14 (22.95)	37 (60.66)
	Ceftizoxime	43 (70.49)	14 (22.95)	4 (6.55)
	Ceftriaxone	31 (50.81)	30 (49.18)	0
	Cefixime	61 (100)	0	0
Carbapenems	Imipenem	6 (9.83)	0	55 (90.16)
Glycopeptides	Vancomyci*n*	0	3(4.91)	58(95.09
Fluoroquinolones	Ciprofloxacin	4 (6.55)	2 (3.27)	53 (86.88)
Quinolones	Nalidixic acid	61 (100)	0	0

In this study, 68.85 percent of isolates were resistant to methicillin and only 1.63% of them were susceptible. In current study, 100% resistance was observed for Cefixime and Nalidixic acid antibiotics. Subsequently, the highest resistance belonged to Cefazolin (96.72%) and cefepime (86.88%). Intermediate Resistance to Vancomycin was 4.91%. The least resistance was observed in two antibiotics Ciprofloxacin and Imipenem with an incidence of 6.55% and 9.83% in isolates, respectively. Since the determination of the susceptibility of all isolates of *Staphylococcus aureus* to Vancomycin cannot be determined by the disk diffusion method, therefore the test of determination of the minimum inhibitory concentration was performed. Vancomycin-resistant *Staphylococcus aureus* isolates were detected by discriminating 30 micrograms of Vancomycin. Identification of isolates that do not show any growth inhibitory haloes should be confirmed. It should be noted, however, that if the Vancomycin inhibition zone is more than 7 mm, then the minimum inhibitory concentration should not be reported as sensitive without testing. The results of electrophoresis of proliferation of antibiotic resistance genes showed fragment lengths of 344 bp and 521 bp, that was related to the presence of *mecA* and *femB* genes in *Staphylococcus aureus*, respectively (Fig. 1 and 2).

The distribution of resistance genes in *Staphylococcus aureus* isolated from the nasal cavity is shown in Table 3. Of 61 isolated *Staphylococcus aureus*, 77.4 percent had the *mecA* gene and 90.16 percent had *femB* gene. 62.29% (38 people) had both *mecA* and *femB* genes.

4. Discussion

Staphylococcus aureus mostly involves children and young adults who are in close contact with the community. This bacterium can cause infections such as necrotic pneumonia and skin infections (David and Daum, 2010). In a study conducted by Wendenberg and colleagues from 91 employees of different departments of the large hospital, 36% of the people were carriers of this bacterium (Vandenbergh and Verbrugh, 1999). In Imam Reza clinical and educational center and Sinaei Hospital Tabriz from 608 patients that had been studied, *Staphylococcus aureus* were isolated from 27.8% patients (Bohlouli et al., 2016). In this study, from 189 patients referred to the Health Center of Amol County, *Staphylococcus aureus* were isolated from 61 cases of them (32.2%).

From these studies, the infection of *Staphylococcus aureus* is increasing dramatically. Also, in this study, 68% of isolates were resistant to Methicillin by disk diffusion method and about 77% by PCR technique, which is lower compared to those obtained in Gorgan, and higher than the Studies took place in Mashhad and Tehran.

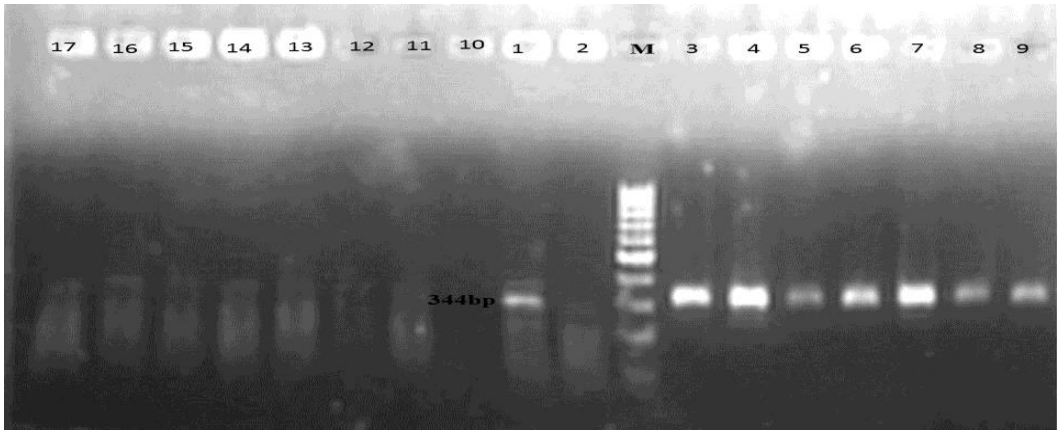

Figure 1. Results of electrophoresis from mecA gene amplification and ladder (M) 1 Kb, lane 1 shows positive control , lane 2 shows negative control, columns 3 to 9 indicate the presence of mecA gene (344 bp) and other columns indicate absence of mecA in Staphylococcus aureus.

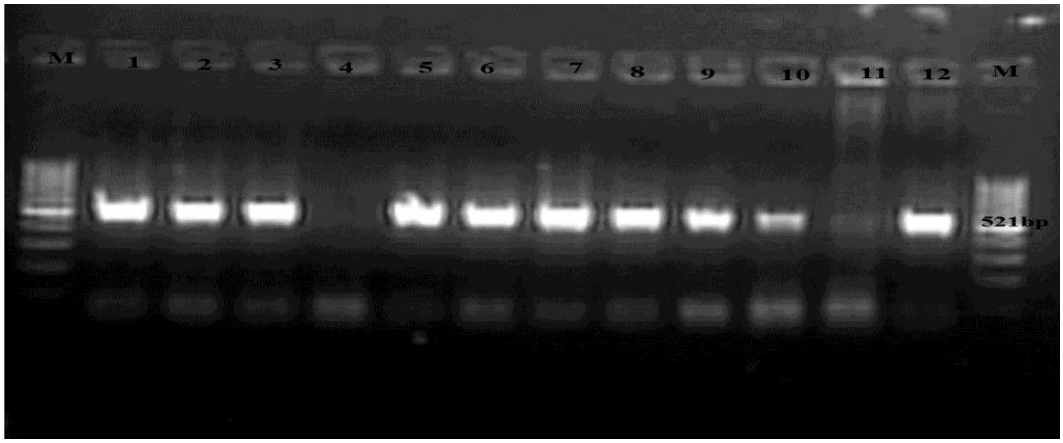

Figure 2. Results of electrophoresis from *femB* gene amplification and ladder (M) 1 Kb, lane12 shows positive control, column 4 show negative control, and columns 5 to 11 and 1to 3 indicate the presence of *femB* (bp 521) gene in *Staphylococcus aureus*.

Table 3. Frequency percentage of genes in isolated *Staphylococcus aureus*

Gene	Positive Number (%)	Negative: Number (%)
mecA	47 (77.04)	14 (22.96)
femB	55 (90.16)	6 (9.84)
vanA	0	61 (100)

The results obtained from PCR of the *vanA* gene have been negative for all isolates.

In this study, the results of the frequency percent of *mecA* and *femB* genes confirmed the results of the propagation of the methicillin resistance disk diffusion method. In a study conducted in Gorgan, resistance to methicillin is reported to be 85%. Frequency of methicillin-resistant isolates in studies conducted in Ireland, Italy and France was less than 50 percent. It seems that excessive use of this antibiotic has led to an increase in methicillin-resistant strains in the community.

The high prevalence of methicillin-resistant strains and also the simultaneous resistance to other antibiotics can have serious consequences for the treatment of infections. In studies by Heo et al in 2007, resistance to methicillin was more pronounced in older adults over 60 years of age (Heo et al., 2007). Along with the regulatory gene of *mecA*, *femB* gene has also been encoded on the chromosome, which affects the resistance level of *Staphylococcus aureus* to methicillin. The results of this study showed that 90.16 percent of the persons had *femB* gene and only 6 (9/84%) had no gene. Glycopeptides are a selective drug for the treatment of infections caused by Enterococci and Methicillin-resistant *Staphylococcus aureus* (Rengarajet al., 2016). In a study of conducted by Rangaraj and colleagues, 109 isolates separated by disk diffusion method showed Vancomycin resistance of 12.9%. In none of the isolates, the *vanA* gene was found. In the present study, with the dilution method, 4.91% of the isolates were intermediate resistance to Vancomycin but none of the isolates the *vanA* gene have been identified. The mechanism resistance is associated with increase in murein synthesis and unavailability vancomycin to penicillin binding proteins. Also, investigate other Vancomycin resistance genes such as *vanB* and *vanC* in these isolates. The results of this study showed a high resistance of this bacterium to Nalidixic Acid and Cefixim, which should be avoided from prescribing these antibiotics. According to a study conducted in Abia in Nigeria state, a total of 70 ear and nose samples were collected. Out of 35 positive strains, 16 hospital and 19 non-hospital strains were investigated for the presence of positive *Staphylococcus aureus*. All isolates showed resistance to Nalidixicacid (Chigbu and Ezeronye, 2003). In this study, the resistance of isolates was 100% to Nalidixic acid. Therefore, alternative antibiotics should be used. Among the reasons for not complying with the results of some researchers and the difference in the frequency of incidence, we can mention the difference in the population studied and the difference in sampling.

Conclusion

In general, this study showed that *Staphylococcus aureus* strains isolated from the nasal cavity are largely resistant to various antibiotics. Considering the high prevalence of antibiotic resistance and different resistance patterns in these strains, in order to treat *staphylococcus aureus* infections and preventing additional costs, as well as controlling the bacterial resistance phenomenon, microbiological tests and sensitivity tests to antibiotic is essential. Regarding the intermediate resistance of *Staphylococcus aureus* to vancomycin and the absence of *vanA* gene, the antibiotic can be used to treat infection caused by the bacterium. Sensitivity to the two antibiotics of fluoroquinolones and imipenem was high in the isolates. Therefore, these antibiotics can be a good treatment option for the treatment of infections caused by this species.

Conflict of interest

The authors declare that there is no conflict ofinterest in this study. This research was supported by a grant from Islamic Azad University, Babol Branch, Iran (Grant No. 12345).

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Refereces

Bohlouli, P., Nahaei, M. R., Farajnia, S., et al. (2016) Vancomycin sensitivity of methicillin resistant *Staphylococcus aureus* (MRSA) isolates collected from clinical specimens of Tabriz Imam Reza and Sina hospitals by E-test method, agar screening plate and PCR. Iranian. J. Med Microbiol. 10(1): 66-75.

Chang, S., Sievert, D.M., Hageman, J.C., et al. (2003). Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. N. Engl. J. Med. 348(14): 1342-1347.

Clauditz, A., Resch, A., Wieland, K.P., et al. (2006). Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. Infec and immun. 74(8): 4950-4953.

Clinical, Institute, L.S., Patel, J.B. (2015). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fifth Informational Supplement: Committee for Clinical Laboratory Standards.

Chigbu Chinwe, O., Ezeronye, O.U. (2003). Antibiotic resistant *Staphylococcus aureus* in Abia State of Nigeria. African Journal of Biotechnology. 2(10): 374-378

Creech, C.B., Kernodle, D.S., Alsentzer, A., et al. (2005). Increasing rates of nasal carriage of methicillin-resistant *Staphylococcus aureus* in healthy children. Pediatr. Infect. Dis. J. 24(7): 617-621.

Cui, L., Ma, X., Sato., K., et al. (2003). Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. 41(1): 5-14.

David, M.Z., Daum, R.S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin. Microbiol. Rev. 23(3): 616-687.

Deurenberg, R.H., Vink, C., Kalenic, S., et al. (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. 13(3): 222-235.

Facklam, R.R., Collins, M.D. (1989). Identification of Enterococcus species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. 27(4): 731-734.

Gould, D., Chamberlaine, A. (1995). *Staphylococcus aureus*: a review of the literature. J. Clin. Nurs. 4(1): 5-12.

Heo, S.T., Peck, K.R., Ryu, S.Y., et al. (2007). Analysis of methicillin resistance among *Staphylococcus aureus* blood isolates in an emergency department. J. Korean. Med. Sci. 22(4): 682-686.

Juyal, D., Shamanth, A.S., Pal, S., et al. (2013). The prevalence of inducible clindamycin resistance among staphylococci in a tertiary care hospital - a study from the garhwal hills of uttarakhand, India. J. Clin. Diagn. Res. 7(1): 61-65.

Malani, P.N., Thal, L., Donabedian, S.M., et al. (2002). Molecular analysis of vancomycin-resistant *Enterococcus faecalis* from Michigan hospitals during a 10 year period. J. Antimicrob. Chemother. 49(5): 841-843.

Noble, W.C., Virani, Z., Cree, R.G. (1992). Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS. Microbiol. Lett. 72(2): 195-198.

Otter, J.A., French, G.L. (2010). Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Europe. Lancet. Infect. Dis. 10(4): 227-239.

Rahimi-Alang, S., Asmar, M., Cheraghali, F., et al. (2011). Frequency of methicillin resistant *Staphylococcus aureus* in health care. Zahedan. J. Res. Med. Sci. 13(1): 17-22.

Ramana, K.V., Mohanty, S.K., Wilson, C.G. (2009). *Staphylococcus aureus* colonization of anterior nares of school going children. Indian. J. Pediatr. 76(8): 813-816.

Rengaraj, R., Mariappan, S., Sekar, U., Kamalanadhan, A. (2016). Detection of Vancomycin Resistance among *Enterococcus faecalis* and *Staphylococcus aureus*. J. Clin. Diagn. Res.10 (2): DC04-06.

Salyers, A.A., Whitt, D.D. (2002). Bacterial Pathogenesis: A Molecular Approach: ASM Press.

Tenover, F.C. (2006). Mechanisms of Antimicrobial Resistance in Bacteria. Am. J. Med. 119 (6, Supplement 1), S3-S10.

Turlej, A., Hryniewicz, W., Empel, J. (2011). Staphylococcal cassette chromosome mec (Sccmec) classification and typing methods: an overview. Pol J Microbiol. 60(2): 95-103.

Vandenbergh, M. F., Verbrugh, H. A. (1999). Carriage of *Staphylococcus aureus*: epidemiology and clinical relevance. J. Lab. Clin. Med. 133(6): 525-534.