ASPI | Afarand Scholarly Publishing Institute; Turkey
ISSN: 2008-2630 *Iranian Journal of War & Public Health* *2023;15(1):43-48* *10.58209/ijwph.15.1.43*

Erythromycin Resistance Genes among Coagulase-negative Staphylococci Isolated from Humans in Basrah, Iraq

ARTICLE INFO

Article Type
Original Research

Authors
Mezban J.M.[1] *MSc,*
Abbas B.A.*[1] *PhD,*
Khudor M.H.[1] *PhD*

How to cite this article
Mezban J M, Abbas B A, Khudor M H. Erythromycin Resistance Genes among Coagulase-negative Staphylococci Isolated from Humans in Basrah, Iraq. Iranian Journal of War & Public Health. 2023;15(1):43-48.

ABSTRACT

Aims In recent years, the global incidence of infections caused by gram-negative bacteria resistant to antibiotics has increased. This study aimed to investigate the presence and frequency of coagulase-negative Staphylococci in contact between animals and people and determine the phenotypic antimicrobial resistance profiles of coagulase-negative Staphylococci isolates from these sources.

Materials & Methods 80 samples were collected from humans in different areas of Basrah Province, including 40 samples from human hand swabs and 40 from nasal swabs. The samples were inoculated onto mannitol salt agar and blood agar and then incubated at 37ºC for 24 hrs. Antibiotic susceptibility testing was performed using the disc diffusion method. A molecular study was done using the PCR technique.

Findings 37 samples (46.25%) were positive for staphylococcal infection. Five species, including S. sciuri, S. lentus, S. gallinarum, S. chromogen, and S. haemolyticus were identified, according to Vitek 2 kit. Staphylococci were resistant to several different antibiotics. Out of 20 amplification samples, only 12 positive samples were purified for the ermA gene region with a PCR product of 190 bp. The results also showed the presence of an ermC band with a size of 299 bp, which represents the correct expected band in 8 isolates out of all isolates.

Conclusion Gram-positive organisms are increasingly identified as the source of acute clinical infection in animals and humans. Some isolates are resistant to several different antibiotics. The ermC gene, ermA gene, and both ermA and ermC genes are present in the genome of these bacteria.

Keywords Erythromycin; Antibiotic Resistance; Bacterial Genes; Staphylococcus; Human; PCR

[1]Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, University of Basrah, Iraq

Correspondence
Address: Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, University of Basrah, Iraq.
Phone: -
Fax: -
basil.abbas@uobasrah.edu.iq

Article History
Received: June 29, 2022
Accepted: January 30, 2023
ePublished: March 1, 2023

CITATION LINKS

[1] Global burden of ... [2] Inactivation of antibiotics ... [3] Co-transfer of ... [4] Survival in maturation ... [5] Antimicrobial resistance ... [6] Antimicrobial resistance ... [7] Identification ... [8] Mobile genetic ... [9] Transfer of ... [10] Methicillin-resistant ... [11] Multiplex PCR ... [12] Detection of enterotoxin ... [13] Iinvestigation of the ... [14] Detection of methicillin ... [15] Isolation and ... [16] The relationship ... [17] Studies on mecA ... [18] Antimicrobial resistance ... [19] Interspecific conjugal ... [20] Antibiotic susceptibility ... [21] Antibiotic resistance ... [22] Sepsis and ... [23] Evaluation of the ... [24] Klebsiella pneumoniae ... [25] Lecture notes ... [26] Erythromycin resistance ... [27] Prevalence of resistance ... [28] Correlation between ... [29] Molecular epidemiology ... [30] Clinical characteristics ... [31] Toxin genes ... [32] PCR-based ... [33] Characterization of enterotoxigenic ... [34] Molecular population ...

Copyright© 2023, the Authors | Publishing Rights, ASPI. This open-access article is published under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License which permits Share (copy and redistribute the material in any medium or format) and Adapt (remix, transform, and build upon the material) under the Attribution-NonCommercial terms.

Introduction

Antimicrobial resistance (AMR) poses a major threat to human health around the world. Many studies have estimated the effect of AMR on incidence, deaths, hospital length of stay, and health-care costs for specific pathogen–drug combinations in select locations. Bacterial AMR occurs when changes in bacteria cause the drugs used to treat infections to become less effective. The Review on Antimicrobial Resistance, commissioned by the UK Government, argued that AMR could kill 10 million people per year by 2050. Although these forecasts have been criticized by some, World Health Organization (WHO) and numerous other groups and researchers agree that the spread of AMR is an urgent issue requiring a global, coordinated action plan to address. Information about the current magnitude of the burden of bacterial AMR, trends in different parts of the world, and the leading pathogen–drug combinations contributing to bacterial AMR burden is crucial. If left unchecked, the spread of AMR could make many bacterial pathogens much more lethal in the future than they are today [1].

The use of antibiotics in humans, to treat infections, and in animals, to promote growth and prevent colonization by pathogenic bacteria, has led to an increased resistance among bacteria [2]. The resistance often is transferable at interspecies and intergeneric levels [3]. The relative ease with which bacteria become resistant to currently used antimicrobial agents is of concern to public health officials [4].

In recent years, the global incidence of infections caused by gram-negative bacteria resistant to antibiotics has increased. It has been predicted that up to two million people in the United States will contract an antibiotic-resistant bacterial infection each year, resulting in over 23,000 fatalities [5].

Staphylococci are gram-positive, non-motile, non-spore-producing bacteria that are ubiquitous and include various opportunistic/pathogenic species responsible for human and animal infections. This group of microorganisms colonizes the skin, hair, nose, and throat of humans and animals, and from these sources, they can be transferred to food because both organisms are the main reservoir [6]. On the basis of the ability to clot blood plasma, Staphylococci are divided into two groups: coagulase negative, and coagulase positive staphylococci [6].

The spread of resistance to antimicrobial agents in staphylococci is largely due to the acquisition of plasmids and/or transposons [7]. In staphylococci, the conjugative transfer of resistance determinants is usually mediated by conjugative plasmids, which spread resistance determinants between species and genera [8]. Besides transferring the resistance determinants, they can mobilize non-conjugative plasmids, recombine with non-conjugative plasmids to form new plasmids, or acquire and transfer resistance transposons [9]. The spread of antibiotic resistance among Coagulase-Negative Staphylococci (CoNS), containing resistance genes, from animal products may represent a hazard to human health through the transfer of resistance genes between staphylococcal species and direct transmission of resistant pathogens to humans [10, 11].

Several previous studies have been conducted on *Staphylococcus aureus* bacteria in the same area [12-17]. Studies with human staphylococcal strains indicate that Staphylococcus epidermidis is a reservoir of antibiotic resistance genes that can be transferred to S. aureus under in vitro and in vivo conditions [18]. Studies of drug resistance transfer between staphylococcal strains have been done mostly on human isolates; studies of transfer between animal and human staphylococcal strains are rare [19].

This study aimed to investigate the presence and frequency of CoNS in contact between animals and people and determine the phenotypic antimicrobial resistance profiles of CoNS isolates from these sources.

Materials and Methods

Samples collection

To obtain Staphylococcus spp., different samples were collected from several regions in Basrah province, Iraq. Forty hand swabs of milker people were collected by moistening the sterilized cotton swab with Buffered Peptone Water (BPW). Swabs were rolled over the palm of the hands, the area between fingers tips, and nails, then incubated for 24 hrs at 37°C. Forty nasal swabs were also collected using sterile swabs. The swab samples were enriched in the appropriate amount of BPW in a 1:9 ratio and incubated at 37°C for 24 hrs according to the standard methods.

Laboratory diagnosis

The specimens were directly transported to the laboratory, then directly inoculated onto Mannitol Salt Agar (MSA) and blood agar, and then incubated at 37 ºC for 24 hrs. All colonies from primary cultures were purified by subculture onto MSA medium and incubated at 37ºC for 24-48 hrs. Gram stain and other biochemical tests were done, such as catalase test, oxidase test, coagulase test, clumping factor test, and hemolysin production. The VITEK 2 is an automated microbiology system utilizing growth-based technology.

Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility testing was performed according to Bauer *et al*. [20] using the disc diffusion method.

Molecular study using Polymerase chain reaction (PCR) technique

Bacterial DNA was extracted using the Geneaid™ DNA Isolation Kit (Bacteria). All staphylococcal isolates were grown in 5 mL of Lysogeny Broth (LB) overnight at 37°C for DNA extraction. Genomic DNA

was amplified using the primers presented in Table 1. These primers were used to amplify the *ermA* and *ermC* genes. From each extracted sample, 5 µl of bacterial DNA was amplified by PCR with specific primers and cycling conditions as previously described [21]. PCR amplified product was detected by electrophoresis on 1% agarose gel. 4 µl of PCR product was inoculated in each well of agarose gel. The molecular weight of the PCR amplified product was determined using a 100 bp ladder after 60 min at 70V.

Table 1) Sequences of primers for *ermA* and *ermC* genes

Genes	Primer sequences	Product size
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCCATTTGGGGAGACT	190 bp
ermC	F: AATCGTCAATTCCTGCATGT R: TTAGCAGTTAAGGACGTACA	299 bp

Findings

Collection and processing of sample

Of the 80 samples collected, only 37 samples (46.25%) were positive for staphylococcal infection, which 19 samples (46.25%) and 18 samples (45.0%) were from human hand swabs and nasal swabs, respectively. There was no significant difference between the type of sample and isolates number (p>0.05; Table 2).

Table 2) Frequency of positive staphylococcal samples according to sample type

Samples type	Number of samples	Positive results
Human hand swab	40	19 (47.5%)
Human nasal swab	40	18 (45.0%)
Total	80	37 (46.25%)
Chi-square (χ^2)=8.922; p>0.05		

Cultural characteristics

Based on culture, suspected colonies of CoNS were smooth, round, raised, glistening, gray to deep golden yellow and white in the color plate, while the colonies on blood agar plates were large, round creamy/buff colored colonies with β or α-hemolysis (Figure 1).

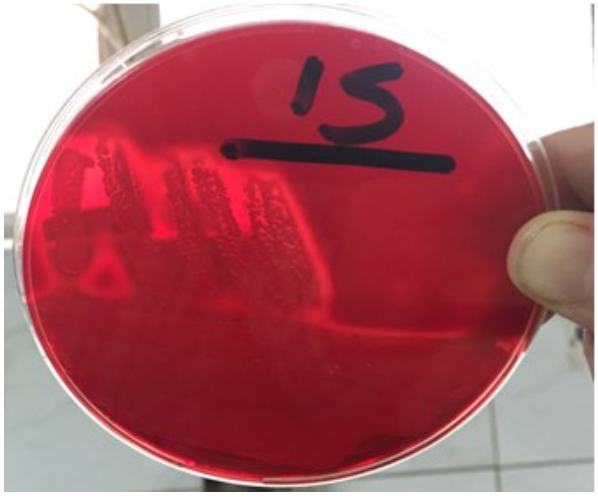

Figure 1) Hemolysis by CoNS on blood agar

With a gram stain, the smear of suspected colonies showed clusters or different irregular shapes of gram-positive cocci. All isolates were positive for the catalase test and negative for oxidase and coagulase tests. There were 5 species of CoNS according to Vitek 2 kit. Staphylococci fell into the following species: *S. sciuri*, *S. lentus*, *S. gallinarum*, *S. chromogen*, and *S. haemolyticus*.

Antibiotic sensitivity of CoNS isolates

37 CoNS from different sources were examined for their susceptibility to antibiotics using the agar dilution method according to NCCLS (National Committee for Clinical Laboratory Standards) guidelines (Figure 2; Table 3).

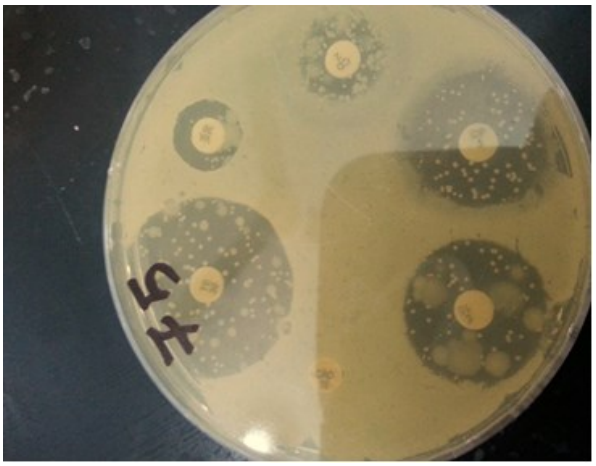

Figure 2) Antibiotic susceptibility test on CoNS

Molecular study

DNA extraction and PCR technique

DNA was extracted from CoNS isolates using conventional methods. The DNA extraction results were accepted, and the concentration and purity were determined using a Nanodrop 1000 spectrophotometer at 280/260 nm. The concentration of DNA was between 135.5-637.7 ng/µl, and the purity was between 1.49 and 2.2, as well as was observed by horizontal gel electrophoresis in 1% agarose (Figure 3).

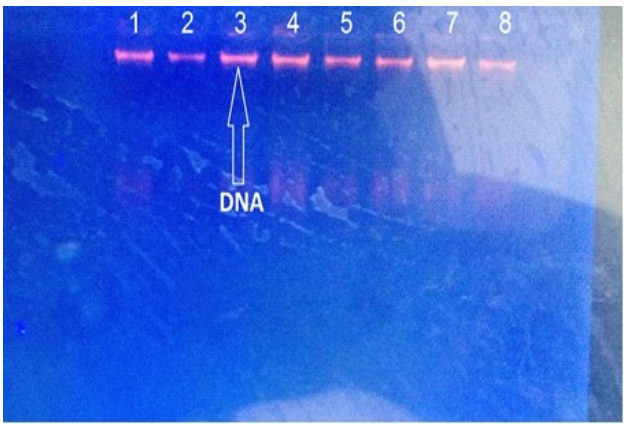

Figure 3) Gel electrophoresis of genomic DNA extraction from some staphylococci
1% agarose gel was observed under UV light at 5 V/cm for 30 minutes.

Detection of *ermA* and *ermC* genes

PCR was performed to detect the *ermA* gene region in 20 staphylococcal isolates, and out of 20 amplification samples, only 12 positive samples were purified for the *ermA* gene region with a PCR product of 190 bp. The results also showed the presence of an *ermC* band with a size of 299 bp, which represents the correct expected band in 8 isolates out of all isolates examined (Figure 4).

Table 3) Antibiotic susceptibility patterns of CoNS isolated during the present study

Susceptibility	AM	AX	ER	CRO	CD	CIP	TE	VA	RA	SXT
Resistant (R)	0	1	2	2	2	6	4	5	7	3
Intermediat (M)	6	5	9	8	6	6	6	7	8	3
Susceptible (S)	9	9	5	7	8	4	7	5	2	9

Abbreviation: AM, Ampicillin; AX, Amoxicillin; ER, Erythromycin; CRO, Ceftriaxone; CD, Clindamycin; CIP, Ciprofloxacin; TE, Tetracyclin; VA, Vancomycin; RA, Rifampin; SXT, Trimthropin/Sulphamethoxide

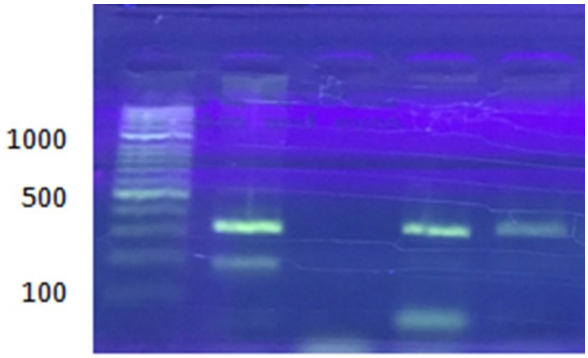

Figure 4) Gel electrophoresis of multiplex PCR products of *ermA* (190 bp) and *ermC* (299 bp) genes of CoNS on 1% agarose gel at 7 V/cm for 1 hour with 100 bp DNA ladder

Discussion

Culturing examination of Staphylococcus was performed by streaking all samples into nutrient agar, mannitol salt agar, and blood agar and incubating at 37ºC for 24 hours. The results showed that most bacteria colonies on nutrient agar, after 24 hours of incubation, were Staphylococcus with an appearance of slightly yellow, flat large, circular, and opaque. When the bacteria grow on the mannitol salt agar, the color of the medium changes. Isolates were gram-positive (purple color) spherical cells or cocci arranged in irregular grape-like clusters. This finding is correlated well with the finding of Fuchs and Sanyal [22], who found that gram-positive organisms have been increasingly identified as the source of acute clinical infection in animals and human.

The VITEK 2 system identify medically important Staphylococci in 15 hours due to a sensitive fluorescence-based technology and allows a result to be generated without the need for a morphological assessment [23].

Most of the isolates were sensitive, and some were intermediate to most of the antibiotics used in the study, as illustrated in Table 3. Resistance may be because some antibiotics cannot penetrate the outer membrane, which may reduce drug permeability.

In Ghostaslo *et al.*'s study [24], the most common organism causing neonatal sepsis was coagulase-negative staphylococci. Gram-negative organisms were isolated in 31.43% of cases, and the most common Gram-negative organism causing neonatal sepsis was *Klebsiella pneumoniae*. They reported that the resistance rates of *K. pneumoniae* to penicillin, ampicillin, cephalexin, chloramphenicol, gentamicin, co-trimoxazol, amikacin, and ciprofloxacin were 94.3%, 91.4%, 82.8%, 74.4%, 60.0%, 54.2%, 40.0%, and 2.8%, respectively.

However, all isolates of CoNS were resistant to ampicillin, corroborating the findings of Elliot *et al.* [25], who documented that CoNS often produce β-lactamases and are resistant to ampicillin and that multiply resistant strains may limit antibiotic choice. The resistance of CoNS to amoxicillin may be due to the common use of this antibiotic in the treatment to most clinical infections.

Six isolates (30%) out of 20 CoNS were positive for the *ermC* gene, and only one sample (5%) was positive for the *ermA* gene, and six isolates (30%) were positive for both *ermA* and *ermC* genes by multiplex PCR method.

Erytromycin resistance in staphylococci is encoded by *erm* genes (*ermA*, *ermB*, *ermC* and *msrA*) [26]. Lim *et al.* [27] reported that the *ermA* gene was more prevalent than the other erytromycin resistance genes in *S. aureus* isolates, and *ermC* gene was found mostly in CoNS. Similarly, in a study performed by Martineau *et al.* [28], the *ermC* gene has been reported to be more prevalent in CoNS. However, *ermA* has been reported to be the more common gene in CoNS in another study [29]. Taponen *et al.* [30] found that the presence of the *ermA* gene is detected in 53.9% of all strains, which is higher than our result (5%), and it disagrees with the present findings.

The ability of CoNS to adhere to extracellular matrix proteins is thought to be essential for the colonization and the establishment of infections [31]. CoNS possesses various adhesion genes, including the *ermC* gene [32].

PCR analysis of the other virulence genes revealed the *ermC* gene in 41 isolates. This finding suggests the important role of these elements in resistance and pathogenicity in bovines and humans. However, *ermC* was present among the strains. Our present results agreed with the combined occurrence of *ermC* genes that has been described by other investigators [33, 34].

Duran *et al.* [21] evaluated the association between antibiotic susceptibility patterns and the antibiotic resistance genes in staphylococcal isolates obtained from various clinical samples of patients attending a teaching hospital in Hatay, Turkey. A total of 298 staphylococci clinical isolates were subjected to

antimicrobial susceptibility testing. The genes implicated in antimicrobial resistance were amplified using the multiplex PCR method, in which a total of 165 isolates were resistant to erythromycin and contained at least one of the erythromycin resistance genes (*ermA*, *ermB*, *ermC*, and *msrA*).

Conclusion

Gram-positive organisms are increasingly identified as the source of acute clinical infection in animals and humans. Some isolates are resistant to several different antibiotics. The *ermC* gene, *ermA* gene, and both *ermA* and *ermC* genes are present in the genome of these bacteria.

Acknowledgements: It is not applicable.
Ethical Permission: Nothing has been reported by the authors.
Conflict of Interests: Nothing has been reported by the authors.
Authors' Contribution: Mezban JM (First Author), Introduction Writer/Main Researcher/Discussion Writer (40%); Abbas BA (Second Author), Methodologist/Assistant Researcher/Statistical Analyst/Discussion Writer (40%); Khudor MH (Third Author), Introduction Writer/Assistant Researcher (20%)
Funding: Nothing has been reported by the authors.

References

1- Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-55.
2- Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science. 1994;264(5157):375-82.
3- Noble W C, Virani Z, Cree R G. Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. FEMS Microbiol Lett. 1992;72(2):195-8.
4- Grabow WOK, Middendorf IG, Prozesky OW. Survival in maturation ponds of coliform bacteria with transferable drug resistance. Water Res. 1973;7(11):1589-97.
5- Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist. 2019;12:3903-10.
6-Anacarso I, Condò C, Sabia C, Messi P, de Niederhausern S, Bondi M, et al. Antimicrobial resistance and other related virulence factors in Staphylococcus spp isolated from food, environmental and humans in Italy. Univ J Microbiol Res. 2013;1(1):1-9.
7- Lozano C, Aspiroz C, Rezusta A, Gómez-Sanz E, Simon C, Gómez P, et al. Identification of novel vga(A)-carrying plasmids and a Tn5406-like transposon in meticillin-resistant Staphylococcus aureus and Staphylococcus epidermidis of human and animal origin. Int J Antimicrob Agents. 2012;40(4):306-12.
8- Malachowa N, DeLeo FR. Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci. 2010;67(18):3057-71.
9- Khan SA, Nawaz MS, Khan AA, Cerniglia CE. Transfer of erythromycin resistance from poultry to human clinical strains of phylococcus aureus. J Clin Microbiol. 2000;38(5):1832-8.
10- Walther C, Perreten V. Methicillin-resistant Staphylococcus epidermidis in organic milk production. J Dairy Sci. 2007;90(12):5351.
11- Mezban JM, Khudor MH, Abbas BA. Multiplex PCR detection of erythromycin resistance genes in coagulase negative Staphylococci isolated from cows in Basrah, Iraq. Bas J Vet Res. 2018;17(1):86-102.
12- Khudor MH, Abbas BA, Idbeis HI. Detection of enterotoxin genes of Staphylococcus aureus isolates from raw milk. Bas J Vet Res. 2012;11(1):254.
13- Abbas BA, Khudor, MH, Idbeis HI. Iinvestigation of the activityand pathogenecity of Staphylococcus aureus enterotoxin c by ligated ileal loopassay in rabbits. Bas J Vet Res. 2013;12(2):104-12.
14- Khudaier BY, Abbas BA, Khudaier AM. Detection of methicillin resistant Staphylococcus aureus isolated from human and animals in Basrah Province/Iraq. MRVSA. 2013;2(3):12-21.
15- Abbas BA, Khudor MH, Hanoon BM. Isolation and identification of Staphylococcus aureus from bovine and the detection of its coagulase gene (Coa) using polymerase chain reaction (PCR). Sc Res Assays. 2014;9(20):864-8.
16- Abbas BA, Khudor MH, Hanoon B. The relationship between biotype, serotype, antibiotic susceptibility and Coa gene polymorphism in Staphylococcus aureus isolated from bovine. Vet Med Assiut Univ Egypt. 2016;17:33.
17- Abbas BA, Khudaier BY, Khudair AM. Studies on mecA gene in methicillin resistant Staphylococcus aureus isolates. Jokull J 2017;58-65.
18- Lyon BR, Skurray RA. Antimicrobial resistance of Staphylococcus aureus: genetic basis. Microbiol Rev. 1987;51(1):88-134.
19- Muhammad G, Hoblet KH, Jackwood DJ, Nielsen SB, Smith KL. Interspecific conjugal transfer of antibiotic resistance among staphylococci isolated from the bovine mammary gland. Am J Vet Res. 1993;54(9):1432-40.
20- Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.
21- Duran N, Ozer B, Duran GG, Onlen Y, Demir C. Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J Med Res. 2012;135(3):389-96.
22- Fuchs M, Sanyal AJ. Sepsis and cholestasis. Clin Liver Dis. 2008;12(1):151-72.
23- Graf B, Thomas A, Edith Z, Göbel UB. Evaluation of the VITEK 2 system for rapid identification of yeasts and yeast-like organisms. J Clin Microbiol. 2000;38(5):1782-5.
24- Ghostaslo R, Ghorashi Z, Nahaei MR. Klebsiella pneumoniae in neonatal sepsis: a 3-year-study in the pediatric hospital of Tabriz, Iran. Jpn J Infect Dis. 2007;60(2-3):126-8.
25- Elliot T, Hastings M, Desselberger U. Lecture notes on medical microbiology. 3rd Edition. London: Blackwell Science: 1997.
26- Weisblum B. Erythromycin resistance by ribosome modification. Antimicrob Agents Chemother. 1995;39(3):577-85.
27- Lim JA, Kwon AR, Kim SK, Chong Y, Lee K, Choi EC. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. J Antimicrob Chemother. 2002;49(3):489-95.
28- Martineau F, Picard FJ, Lansac N, Menard C, Roy PH, Ouellette M, et al. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic suseptibility patterns of Staphylococcus aureus

and Staphylococcus epidermidis. Antimicrob Agents Chemother. 2000;44(2):231-8.
29- Thakker-Varia S, Jenssen WD, Moon-McDermott L, Weinstein MP, Dubin DT. Molecular epidemiology of macrolides-lincosamides-streptogramin B resistance in Staphylococcus aureus and coagulase-negative staphylococci. Antimicrob Agents Chemother. 1987;31(5):735-43.
30- Taponen S, Simojoki H, Haveri M, Larsen HD, Pyörälä S. Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Vet Microbiol. 2006;115(1-3):199-207.
31- Akineden, Annemüller C, Hassan AA, Lmmler C, Wolter W, Zschck M. Toxin genes and other characteristics of Staphylococcus aureus isolates from milk of cows with mastitis. Clin Diagn Lab Immunol. 2001;8(5):959-64.
32- El-Sayed A, Alber J, Lmmler C, Bonner B, Huhn A, Kaleta EF, et al. PCR-based detection of genes encoding virulence determinants in Staphylococcus aureus from birds. J Vet Med B Infect Dis Vet Public Health. 2005;52(1):38-44.
33- Stephan R, Annemüller C, Hassan AA, Lmmler C. Characterization of enterotoxigenic Staphylococcus aureus strains isolated from bovine mastitis in north-east Switzerland. Vet Microbiol. 2001;78(4):373-82.
34- Fitzgerald JR, Hartigan PJ, Meaney WJ, Smyth CJ. Molecular population and virulence factor analysis of Staphylococcus aureus from bovine intramammary infection. J Appl Microbiol. 2000;88(6):1028-37.