Article Review: Virulence Factors of Coagulase-Negative Staphylococci Isolates from Iraqi Patients

Rana H. Raheema1, Ban H. Qaddoori2 and Marwa A. Al-Asady3
1Department of Medical Microbiology, Collage of Medicine, University of Wasit, IRAQ
2Department of Medical Microbiology, Collage of Medicine, University of Wasit, IRAQ
3Department of Medical Microbiology, Collage of Medicine, University of Wasit, IRAQ

1Corresponding Author: rraheema@uowasit.edu.iq

ABSTRACT

The aim objective of this research is a review study of the virulence factors of coagulase-negative staphylococci (Co.N.S) species isolated from Iraqi patients. The world is now facing a major problem between microbes and antibiotics. The most important of these microbes are Co.N.S species and they are one of the opportunistic microorganism and considered one of the causative agent of nosocomial infection, bloodstream infections (especially in neonatal sepsis and catheter-related bloodstream), urinary-tract infections, mastitis, wound infections and ear/eye infection. Where these bacteria are notable by their resistance to various antibiotics by produce β-lactamase, present of efflux-mechanisms pumps, increase thickness of cell well or by horizontal transfer of resistance genes (plasmid) from other bacteria of the same or different species.

The too much use of antibiotics, led to the resistance of microbes to these antibiotics and is the major cause of the current problems of the world, as microbes have the capability to develop mechanisms that allow them to overcame these antibiotics more rapidly and difficult to find choices to these antibiotics.

As well as having an important virulence factor is biofilm (polysaccharide intracellular adhesion) that resists phagocytosis and primarily responsible for chronic infections because of their resistance to phagocytosis and killing due to cellular/ humoral immunity. In addition, having toxin, protease, hemolysins, urease, lipase and catalase help them to colonization/invasion the host tissue and evasion the host immunity.

Keywords: Coagulase-negative Staphylococci, Virulence factors, Antibiotics resistance.

I. INTRODUCTION

Nowadays, as traditional opportunists and one of the main nosocomial agents, coagulase-negative staphylococci (Co. N.S), are of significant impact on human life and health (Solati et al., 2015; Shrestha et al., 2017). The number of resistant strains, including penicillin, oxacillin, methicillin, clindamycins, erythromycins, ciprofloxacins and gentamicins has increased dramatically and histronically. Increased resistance to antimicrobial medicines in Co. N.S restricts the treatment option (Obajuluwa et al., 2017). Many species are included in Co. N.S. The most common Co. N.S colonizing of human skins and mucous membranes is staphylococcus epidermidis, which causes nosocomial infections. Resistance to antimicrobials in S. Over the last couple of decades Epidermidis has expanded considerably, threatening human health globally; biofilms can also be produced making it hard to eradicate (Talat et al., 2020; Al-Asady et al., 2020). Additional Co. N.S species are S. hominis, S. xylosus, S. lentus, S. haemolyticus, S. lugdunensis.

The whole S. epidermidis genome was studied in a research carried out by Talat et al. the Iraqi patient, with an ear infection, was isolated from the S. epidermis AK-612, and its multidrug resistant phenotype was investigated. The MLST ST35 S. epidermid of AK-612, previously recorded only in Portugal and German, was identified as ST35 of the CC2. Replication proteins RepL (Plasmid-Maintenance Protein (Node 5), REB (Plasmid-Family Replication Protein Initiator (Nodes 12,18), Replication Protein RepB (Node 31), RRI (Node 31), RTP (Node58), and RI (Node 62) were recorded in the genome. Sensitivity testing for antimicrobials has shown that S. epidermidis AK-612 resistant to imipenem ampicillin, aztrimam, and cefoxitin. S. epidermidis acquired genes for antimicrobial resistance included: fosB providing fosfomicin resistance; blaZ and mecA conferring β-lactam resistance; fusB conferring fusidic acid resistance; mphC conferring macrolide resistance; and msrA conferred lincomamides resistance and streptomycin B resistance. Predicted genes CARD have been predicted to avoid trimethoprim (gene dfrC), streptogramines (gene msrA), fusidic acid (gene fusB), β-lactams (gene blaz), erythromycin, quinolones (gene norA), tetracycline (gene mgra), methicillin (gene mecA), and macrolides (gene mphC). The mecA gene that codes a penicillin binding protein (PBP-2A) with low affinity for antibiotics of β-lactam is associated with methicillin resistance and is therefore established as S epidermis resistant to methicillin. (MRSE). The protein A is also lysostaphin immune in the genome. The pathogen finder predicted S. epidermidis AK-612 to be a 0.948 human disease pathogen. The proteins combined with 331 pathogenic, but non-pathogenic, families are interesting. The genome was distributing ISSepI of the IS1182 family, the ISSau4 of the IS3 family, the IS257R1 of the IS6 family, the...
IS431 mec family IS6, the MITE Sen1 family IS256, the IS38 family IS110 and the IS20 3 family IS200 / IS605. Methicillin resistance is given by IS431 mec family IS6. The presence of multi-medical resistance genes, virulence factors, SCCmec type V and mobile components as IS256 and IS A31 mec makes this line a potential challenge to the development of efficient transmission dynamics by manipulating its plasmid and transposons. Co. N.S contains multiple virulence factors that facilitate host tissue colonization, immune system evasion and antibiotic resistance.

II. THE VIRULENCE FACTORS

Biofilm

The biofilm helps microbes in pathogenicity by protecting them from phagocytosis, chemotaxis, and antimicrobial agents. In Co. N.S biofilm creation, mediated by polysaccharides intracellular adhesion (PIA) production, is considered the essential virulence factor of Co. N.S (Qin et al., 2017). PIA is encoded by the intracellular adhesion (ica) locus, involving of the icaADBC structural and icaR regulatory genes. Amongst them, the icaA and icaD genes play a central role in the accumulation stage of biofilm production (Arciola et al., 2002; Limoli et al., 2015). FnB A gene is a fibronectin-binding protein, promotes biofilm formation through the binding of the protein to a surface-located receptor on adjacent cells (Herman-Bausier et al., 2015). Aae is a surface-associated protein with bacteriolytic and adhesive properties representing a new member of the staphylococcal autolysin/adhesins potentially involved in colonization (Morello et al., 2006). The atlA (autolysin), which has an adhesive function that is involved in the first phase of biofilm formation. The expression of the atl gene increased in the vancomycin resistance (Heilmann et al., 2003).

In a study conducted by Al-Asady et al. (2020) one hundred and ninety-two samples were collected from different hospitals and clinics private in Wasit province. 89 samples were obtained Co. N.S include S. epidermidis (36) and S. hominis spp. hominis (16), S. xylosus (10), S. lentus (6), S. auricularis (5), S. warneri (4) and S. capitis (3), S. cohni (2), S. lugdunensis (2), S. sciuri (2), and S. haemolyticus (1) isolate. S. epidermidis (36 isolates) was the predominant species among Co. N.S. 30 isolates of S. epidermidis obtained to biofilm-forming and 6 non-biofilm forming by MTP method. The detection of virulence genes of S. epidermidis biofilm-forming showed that 26 isolates were harbored icaA and icaD gene, 29 isolates were harbored fnbA gene. Whereas no isolates have bap gene. These isolates showed resistance to most of the antibiotics such as cefepine, ciprofloxacin, doxycline and levofloxacin. S. epidermidis has the ability to produce biofilm making it difficult to eradicate. Another study conducted by Nezar et al. (2020) found from 56 nipple discharge from non-lactating women in wasit province, 40 isolates as S. epidermidis. The genotype icaA, icaD, mecA, and sei were used. The results demonstrated that 6/40 have icaA, 40/40 have icaD, mecA 38/40, and sei enterotoxin 6/40. These isolates showed resistance to cefpodoxim, cefixime, vancomycin, amoxicillin/clavulanic acid, cefotaxime, and ceftriaxone.

In a study by Abdraba and Flayyih, (2019) they collected thirty S. epidermidis isolates from clinical specimens from patients attending Baghdad Teaching Hospital, Laboratory teaching of Baghdad medical city, and AL-kindly teaching Hospital. The results showed that 12 isolates were (VRSE), 4 isolates (VISE), and 14 isolates (VSSE). Used PCR to detect the virulence factors (autolysin, adhesion), phageflatococal surface protein, aae gene in S. epidermidis. All S. epidermidis isolates gave a positive result for the presence of aae gene. The results revealed that was a significant difference among three isolates, the VSSE isolate has the highest autolytic activity in the presence of an antibiotic, followed by the VRSE isolate and the VISE isolate, which was the lowest autolytic activity with the presence of the antibiotic. The result of the transmission electron microscope showed that the VRSE isolates have a thicker cell wall followed by VISE isolates. However, the KSSE did not show any cell wall thickening. Autolysis is linked to the process of cell division and is therefore related to the growth of the cell and the expression of autolysins, which hydrolyses cell wall components. The autolysis rates of S. epidermidis with induced vancomycin resistance were less than those of the sensitive isolate. This suggests that resistance to autolysis indicates a reduction in cell wall turnover.

In another study by Baqer and Mahdi, (2019) 247 specimens (breast milk) were collected from lactating women, 147 lactating women with mastitis, and one hundred from healthy lactating women, from Baghdad and Al-Kadhimiya Teaching Hospitals. Seventy-four Co. N.S isolates were detected (56 from mastitis cases and 18 from healthy women). The most predominant bacterial isolates in lactating women (mastitis and healthy) were S. epidermidis 25/56 and 9/18 respectively. The highest resistance percentages were found towards ampicillin and erythromycin 100%, trimethoprim/sulphamethoxazole 98.52%, methicillin 97.05%, carbencillin 95.58%, gentamicin 72.05%, cefotaxime 61.76%, temocillin 60.29%, ceftazidim 58.82 %, cepfime 55.88%, ciprofloxacin 54.41%, ampicillin/sulbactam 52.94%, 52.94 %, 48.52 % and 45.58% of all S. epidermidis isolates were resistant to cefoxitin, nalidixic acid and tobramycin, respectively. In addition, 33 isolates of S. epidermidis industrialized multidrug resistance, and all of S. epidermidis isolates were found to be biofilm producers. The resistance to diverse antibiotics and a higher ability to form biofilms, found among the strains isolated from milk of women suffering mastitis, might explain the chronic and/or recurrent nature of this infectious condition.
Al-Kalidy and AL-Hasnawi (2019) collected 60 isolates Co.N.S from patients and carries (40 of clinical origin and 20 of carries origin) in Diwaniyah Teaching Hospital, Hakim Teaching Hospital, and Zahra Hospital Maternity and Children in AL-Najaf province. Seven species were identified. All species were present in clinical isolation while only five species in carries isolation. The species included; S. epidermidis (18 isolates), S. saprophyticus (13 isolates), S. haemolyticus (11 isolates), S. hominis (7 isolates), S. lentus (6 isolates), S. capitis (4 isolates) and S. auricularis (1 isolate). S. epidermidis was the Co.N.S species most frequently isolated from clinical samples, corresponding to (10 isolates). The remaining species were distribution among S. saprophyticus (9 isolates) S. haemolyticus (8 isolates). S. hominis (3 isolates), S. lentus (5 isolates), S. capitis (4 isolates) and S. auricularis (1 isolate). In carry isolates, S. epidermidis was the dominating species (8 isolates) followed by S. saprophyticus and S. hominis (4 isolates) from each species while (3 isolates) S. haemolyticus and one isolates to S. lentus. The result indicated no significant difference between the clinical and carries Co.N.S isolates (P>0.01). Results of gene amplification by multiplex PCR showed the difference between clinical and carries isolates was statistically significant for the presence of the mecA & icaA genes (P≤0.01). 30 clinical isolates expressed the mecA gene with the highest percentage in S. epidermidis and S. haemolyticus while only 7 carries isolates expressed the mecA gene. 12 isolates expressed the icaA gene of clinical isolates while only 2 of carries isolates expressed the icaA gene. The lack of the mecA gene in biofilm negative phase variants suggests its possible role in pathogenicity. The development of resistance to multiple drugs in Co.N.S is important and is related to biofilm production and its growth pattern.

In 2013, Al-Mulla et al. obtained 87 staphylococcal isolates from wound swabs, ear swaping and urine samples of patients in Baghdad City's various hospitals (Baghdad Hospital, Baghdad City Hospital, AL-Yarmook Hospital and Ibn Al-Baladi Hospital). Co. N.S (S. epidermidis) was acquired for 65 isolates. 14% of these isolates were listed as producers of biofilms.

Isolates of S. epidermidis were identified in a study carried out by Ismail et al. (2011) in Ibn Al Haietham Eye Hospital, Baghdad among 57 patients. A total of 37 S. epidermidis isolates were tested for slime production, 52.63% were positive-slime production of all isolates (23 isolates in patients and seven isolates in controls) from the corneal scraping of keratitis patients and twenty healthy eye isolates. Rifampin and chloramphenicol were extremely resistant to the isolates. It was found that S. epidermidis produces positive slime. In comparison to the negative slime isolates, epidermidis had high resistance to antibiotics.

**Enzyme**

Most strains of Co.N.S that produce exoenzymes, which probably contribute to the persistence of Co.N.S are in the host and can damage host tissue. Lipase enzymes contributing to virulence, it’s important for the colonization and persistence of resident organisms on the skin, possibly in terms of nutrition or by the release of free fatty acids which may promote adherence (Otto, 2004). The hemolytic activity has been discovered in some Co.N.S strains, alpha hemolysins have been related to neurotoxic action and delta hemolysins with a severe inflammatory response, and damage in a variety of mammalian cells (Michelim et al., 2005). Proteases have three different catalytic classes, including metallo, serine, and cysteine enzymes that are found among the secreted staphylococcal proteins. The serine protease is especially stated in adherent culture, suggesting a probable role in biofilm development (Reed, 2007). Urease enzyme is an essential virulence factor of S. saprophyticus and other Co.N.S species maybe contributes to intrusiveness by the destruction of bladder tissues and causes urinary tract infections (Lina et al., 2000).

A study collected 150 urine specimens from urinary tract infected patients who visited various hospitals in Baghdad by Al-Mathkhury et al. found 48 isolates obtained Co.N.S. From the Co.N.S (16.3% isolates) suggested being S. xylosus. Also, S. cohnii, S. lentus, and S. saprophyticus were isolated with percentages of 5.6%, 2.4%, and 13.5% respectively. The isolates of S. xylosus could produce protease and hemolysin, but they could not produce lipases while the activity of urease was variable. The urease produces isolates S. xylosus was evident more virulent than the non-urease producing isolates S. xylosus and the isolates have demonstrated high erythromycin resistance.

A research by Omran and Hussein (2009) stated that 33 Co. N.S included S. lentus (20 isolates) and S. xylosus, respectively. Clinical cases and healthy carriers S. xylosus (13 isolates) from the AI Diwanyah hospital teachers. Out of 33 isolates, 13 isolates of S. lentus and 7 isolates of S. xylosus were resistant to methicillin. Twenty isolates (13 S. lentus, 7 S. xylosus), immune to methicillin and oxacillin, were investigated into hemolysin activities.

Moreover, 13 isolates of S. lentus were urease producers. The similarity between clinical and carrier isolates is expressed by the mecA gene with a high percentage in S. lentus and S. xylosus. 20 isolates carried mecA genes, represented by 13 S. lentus and 7 isolates of S. xylosus. A good coloration was observed between phenotypic oxacillin susceptibility testing results and PCR amplification results for Co. N. S.

**Toxin**

Staphylococcal superantigens (SAGs) including staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TST1) (Park et al., 2011). Twenty-three different staphylococcal enterotoxins (SEs) have been described, including SE-A to SE-V. All enterotoxin share super antigenic activity, whereas, only a few of them (SE-A to SE-I, SER, SE-S, and SE-T) has been verified to be emetic (Larkin et al., 2009). The
enterotoxins generated by the sea gene generate more severe immunological responses and subsequently more tissue damages compared with other enterotoxins (Ferry et al., 2005). The enterotoxin genes are encoded in mobile genetic elements, such as prophages, plasmids, and pathogenic islands. Those mobile genetic elements are responsible for the horizontal transfer of virulence or antibiotic resistance genes (Nunes et al., 2017).

In study by Hussein et al. (2019), different clinical samples 251 were collected from Al-Musayib General Hospital in Babylon, Iraq. 60 isolates were Co.N.S include S. sciuri (31.66%), S. haemolyticus (25%), S. saprophyticus (23.33%), and S. lentus (20%). All isolates showed resistance to mexitillin antibiotic (phenotypic method). In the genotypic method to detect methicillin resistance isolates was done depending on a mobile genetic element mecA, a multiplex PCR assay was intended for direct detection of methicillin resistance gene mecA. The results showed that 25% of isolates contained the mecA gene. To detect the distribution of Super antigenic toxin genes among the isolates used the isolated, seb, seg, seh, sej, selp, and tsf genes. Results showed that only S. sciuri carry seb, seh genes, S. lentus carry seh gene, S. saprophyticus carry seg, sej, tsfl genes, S. haemolyticus avoid any of these genes. AL-Hadithi et al. recorded 15 isolates of methicillin-resistant coagulase-negative staphylococci (MR-Co.N.S) from 15 cases of bacterial conjunctivitis. All isolates were resistant to penicillin followed by tetracycline and cloxacillin (88.7%). Only one isolate was resistant to vancomycin. Screening isolates for growth on vancomycin-containing media has revealed that 6 out of 15 isolates (40%) exhibited intermediate resistance to vancomycin (VI-Co.N.S). Only one isolate (6.7%) was found completely resistant to vancomycin (VR-Co.N.S). All vancomycin-resistant strains (six VI-Co.N.S, one VR-Co.N.S) were found to produce biofilm (100%).

III. MECHANISM OF ANTIBIOTICS RESISTANCE

Antimicrobial resistance may be acquired through mutation and selection of resistant bacterial strains or horizontal transfer of resistance genes from other bacteria of the same or different species (Chambers & De Leo, 2009). Increasing antimicrobial drug resistance in Co. N. S limits the therapeutic choices. The common resistance mechanisms in Staphylococcus are the production of enzymes that inactivate or destroy the antibiotics, efflux-mechanisms pumps, and decrease of the antibiotic binding affinity to the drug (Lenart-Boron, 2016). A beta-lactamase is an important enzyme that produces by bacteria and that increases the resistance of bacteria to antibiotics. 

Beta-lactamase & mecA gene

Resistance to β-lactams, that is, MR-Co.N.S (methicillin-resistant Co.N.S) is determined by the presence of mecA gene (Singh et al., 2016). The mecA gene, which encodes a PBP-2a, with reduced affinity for methicillin compared with the attractions of other PBP. In addition to methicillin resistance, Co.N.S strains have acquired resistance to several other antibiotics including rapamycin, fluoroquinolones, gentamycin, tetracycline, erythromycin, chloramphenicol, clindamycin, and sulphonamides (Rogers et al., 2011). The research by Shaker and Lafta (2019) collected different specimens (biopsy n=50, swab n=50) from women patients with malignant and benign at Baghdad Medical University Hospital in Baghdad, Iraq. From these samples, 39 isolate was S. epidermidis (27 malignant and 12 benign). S. epidermidis isolates showed multi-drug resistance. Most isolated bacteria from benign and malignant tumors were resistant to Oxacillin, Cefixime, Penicillin, and Meticillin. In addition, an accurate PCR test was carried out here for the identification of the isolates using primers specific to the S. epidermidis recN gene, which encodes a repair and recombination protein. This gene could be used to forecast whole-genome relatedness with high accuracy. The mecA gene was amplified in S. epidermidis isolates. All isolates except one have mecA and hence were methicillin-resistant. But this does not exclude the ability of this bacterium that lacks mecA from being pathogenic.

A study by Ali et al. collected 265 samples taken from patients in Tikrit General Educational Hospital. The results revealed 60% Co. N. S, which shows 27 isolates regards S. epidermidis , 13 isolates S. saprophyticus , 10 isolates S. xylosus , 3 isolates S. lentus, 2 isolates S. haemolyticus and one isolate S. simulans, and another one isolate of S. hominis. The research made a test for producing β-lactamase enzymes, that show 85% of isolates were positive to this test because the β-lactamase enzyme is one of the significant enzymes which are produced by bacteria since it improves the resistance of bacteria for antibiotics.

In a study, done by Taha et al. 30 clinical isolates of S. haemolyticus were isolated from blood cultures of neonate patients at a hospital in Baghdad. All isolates showed a high level of resistance to oxacillin and benzylpenicillin (100%). Phenotypic detection test of methicillin resistance to S. haemolyticus isolates showed all 30 S. haemolyticus isolates under test were methicillin resistance and the results of PCR assay for detection of mecA gene showed from 30 isolates of methicillin-resistant S. haemolyticus, 28 isolates were positive to mecA gene while only 2 isolates were negative to mecA gene.

Another research by Aldeen and Méshkoor, in (2017), included breast milk samples of 200 mastitis patients and 106 local women visiting Al-Sadder hospital in Najaf, Iraq. Sixty two breast milk isolates (50 mastitis, 12 control), identification of Co.N.S at the species level indicated that S. epidermidis was the most common species, with 40 isolates, followed by S. haemolyticus (10), S. hominis (12). It is noticed that most of Co.N.S (56/62) isolates were resistant to penicillin G, Cefoxitin, https://doi.org/10.31033/ijrasb.7.5.49
oxacillin. All isolates appeared to have mecA gene, no one harbored SCCmec type I, 8 harbored SCCmec type II, 12 harbored SCCmec type III, 30 harbored SCCmec type IV and 8 remained non-type able. S. epidermidis was the most isolates that harbored SCCmec type IV.

A review of Al-Charrakh and Obayes, (2014) obtained from 690 samples in four health centers in Al-Hilla, Iraq, from patients suffering from a variety of infections. 178 isolates of Co.N.S have been restored. 10 separate species; 22 isolates were identified as S. lugdunensis. In 15 out of 22 isolates that were phenotypically identified, S. lugdunensis was found. The other seven isolates have been reconfigured as S. pseudolugdunensis. Furthermore, a suitable S. lugdunensis diagnosis nucleic acid target. S. lugdunensis is a fbl gene encoding the binding adhesion of the S. lugdunensis. The gene in all 15 S. lugdunensis has been detected. This means fifteen isolates have been found positive for S. lugdunensis tanA and fbl genes. The blackjack. The results suggested that 11 isolates from S. lugdunensis were mainly screened for β-Lactam resistant isolates. Also, S. lugdunensis was ampicillin resistant. In the presence of ampicillin, all these isolates were natural to develop, and this is recognized in most S. lugdunensis isolates. Oxacillin-resistant screening findings revealed that 7 S. lugdunensis isolates resistant to 11β-lactam were oxacillin-resistant. Results showed that 11 of 15 S were susceptible to β-Lactam Antibiotics. The isolates from S. lugdunensis were high in ampicillin resistance (73.3%). The findings also demonstrated that the resistance rate of cephalotin, ceftriaxone and cefexime to oxacillin and cloxacillin stood at 46.6 percent. For these antibiotics, S. lugdunensis resistant isolates were significant: 46.6%, 53.3%, and 40%, respectively, amoxiclav 60%, and ceftazidime / clavulanic acid 53.2%, and imipenem-prone isolates (80%). Six isolates of oxacillin resistance had a mecA gene, but the same gene did not occur in one isolate. Of the 15 isolates of S. lugdunensis, 14 were vancomycin-sensitive, while only one was less susceptible to vancomycin (interim). The producers of β-lactamase were eight isolates (53.3%). All these isolates were resistance to ampicillin; seven of eight β-lactamase isolates developing were resistant to oxacillin, while the remainder was oxacillin-resistant. Six out of eight had mecA.

In another study by Ali et al. (2009) sixty isolates of Co.N.S were collected from patients at Tikrit teaching Hospital, were 26 isolates belong to S. epidermidis, 13 isolates S. saprophyticus, 10 isolates S. xylosus, 3 isolates for each S. sciuri and S. lentus, 2 isolate S. haemolyticus, one isolate for each S. simulans and S. hominis. The antibiotic susceptibility test showed the most effective antibiotics was nitrofurantoin (95.83%), followed by amikacin (93.33%), novobiocin (90.83%), chloramphenicol (88.33%), rifampicin (79.16%), vancomycin (75.83%), ciprofloxacin (74.16%), clindamycin (72.5%), gentamicin (61.66%), penicillin G (49.16%), trimethoprim (49.16%), tetracycline (40%) and cefoxitine (17.5%). In addition, the beta-lactamase test was performed, and the results have shown 85% of isolates were positive beta-lactamase test.

**Van A and van B gene**

The van A gene plus several additional genes and stains that carries plasmid required for vancomycin resistance. The proteins encoded by these genes are responsible for lowering the cell-wall affinity for vancomycin (Sibbald et al., 2012). In a study done by Flayyih and Abdrahaa, (2015) they reported out of 100 clinical samples, collected from patients attending to Baghdad teaching Hospital, Laboratory teaching of Madienat AL–Teb, and AL–Kindy teaching Hospital, 50 isolates belong to Co.N.S, in which the last S. epidermidis isolates were 30. The results revealed that high resistance to penicillin G10 and amoxicillin-clavulanic acid 100%, aethicillin were (93%), erythromycin (90%), gentamycin, ceftazidime and clindamycin (70%), tetracyclin (75%), ciprofloxacin (60%). Results of vancomycin sensitivity test shown that from 30 isolates, 12 isolates were resistant to Vancomycin, 4 isolates were intermediate resistant and 14 isolates were sensitive. The result of this study showed that some isolates contain one large plasmid (megaplasmid) and other contain two plasmids and three plasmids among vancomycin-resistance S. epidermidis (VRSE) and vancomycin-intermediate resistance S. epidermidis (VISE), one isolate among vancomycin-resistance S. epidermidis (VRSE) contain three plasmids, four isolates contain two plasmids and sixteen isolates have one plasmid, but nine isolates of vancomycin-sensitive S. epidermidis (VSSE) don’t contain any plasmid. The accurate and rapid diagnosis of antibiotic resistance genes in the treatment of S. epidermidis infections is extremely important in preventing the spread of infections. The genetic determinants of vancomycin-resistant vanA and vanB were amplified to identify susceptible. The results of this study showed that vanA gene bands were detected in 12 S. epidermidis isolates but non-S. epidermidis isolates were produced by vanB gene bands.

**Efflux pump**

Tetracycline resistance in staphylococci can progress by two mechanisms either ribosomal or efflux protection. Efflux is generally encoded by tetK found on transposons or plasmids frequently combined within the SCCmec elements. The ribosomal protection encoding tetM gene is chromosomally located (Ardic et al., 2006; Grundmann et al., 2011). In study by Barzani et al. (2016), 32 isolates of Co.N.S was isolated and identified from clinical sources; including 8 isolates of each of S. hominis and S. haemolyticus from (64 patients with UTIs) and 8 isolates of each of S. epidermidis and S. auricularis from (50 patients with otitis media infection) which suffering in Rizgary teaching hospital in Erbil city. The isolates showed high resistance to tetracycline with a percentage of 50%, whereas they differ in their resistance to other remaining antibiotics, which were oxacillin,
teicoplanin, piperacillin, cefoxitin, cephalothin, ofloxacin, and ceftazidime. On the other hand, all isolated S. hominis, S. haemolyticus, S. epidermidis, and S. auricularis isolates were tested for tetracycline-resistant genes tetK and tetM used PCR. PCR method indicated that 75% of S. hominis, S. haemolyticus, and S. epidermidis were carried tetM gene, while 50% of S. auricularis was carried the tetM. It means that the tetM was found also in some sensitive isolates to tetracycline phenotypically. On the other hand, it was found that 50% of S. hominis and S. haemolyticus isolates were harboring tetK gene and no tetK gene was observed in S. epidermidis and S. auricularis. Al-Salmani et al. study conducted in Baghdad Medical City / Child Welfare Teaching Hospital, obtained 46 isolates of S. haemolyticus from blood crops. The resistance of only two isolates to linezolid was at all concentrations of linezolid used (i.e. 2 μg/ml, 4 μg/ml, 8 μg/ml and 20 μg/ml). The vulnerability of ten representative isolates (8 line-solid and 2 resistant), which appeared to occur as a result of methylation mechanisms in 9 isolates and isolates due to another mechanism, indicates the highest reported erythromycin resistance of 10 (100 per cent), respectively. 10 isolates. Clinamycin and cloramphenicol resistance has been reported. The msrA gene, which codes an ATP dependence-efflux pump, gives resistance to erythromycin.

IV. CONCLUSION

Through a review of many research studies of the Iraqi isolates of Co.N.S, it became apparent that the ability of these bacteria to resist antibiotics increases through the development of the virulence factors particularly that used against staphylococcal infection. This indicator shows the difficulty of determining new antibiotics that work to destroy/kill these bacteria therefore, probiotics, probiotic and postbiotic have been used recently to inhibit them.

REFERENCES

[34] Park, J.Y. et al. (2011). Detection of classical and newly described staphylococcal superantigen genes in coagulase-negative staphylococci isolated from bovine intramammary infections. Veterinary Microbiology, 147, 149-154.