Isolation of Methicillin-Resistant Coagulase-Negative Staphylococci from Hemodialysis Patients in Al-Najaf Province

Oday Mitib Hadi[1], Ihsan Khadum Humaidy[2]

[1]Professor Assistant, Medical Laboratory Techniques Department, College of Health and Medical Techniques-Kufa, Al-Furat Al-Awsat Technical University, [2]Lecturer Assistant, Al-Sader Medical City, Najaf Health Department, Najaf- Iraq

Abstract

Information about methicillin-resistant coagulase-negative staphylococci (CoNS) is unavailable in our province, in comparison with methicillin-resistant *Staphylococcus aureus*. Two hundred and forty isolates of Staphylococci were recovered from hemodialysis patients, from July-2019 to January-2020. Antimicrobial susceptibility was performed for methicillin-resistant CoNS, by using the Kirby and Bauer technique. *mecA* gene was detected by polymerase chain reaction (PCR). The results revealed that the infections were predominant in males (66.7%) especially in the age group more than 60 years old. Patients from urban areas occupied the highest percent in comparison with the rural areas.

Staphylococcus epidermidis was predominant among other isolated genera (63.33%), of these forty strains (26.32%) were *mecA* positive, while *Staphylococcus saprophyticus* takes only (16.67%) and six isolates were appeared to be *mecA* positive. Resistant patterns illustrated that most *mecA* positive strains were resistant to more than one antibiotic of none-beta lactam antibiotics especially rifampin (60.87%).

In conclusion, the increase of the antibiotic-resistant by coagulase-negative staphylococci, and in particular *mec A* gene containing CoNS, is a risk factor for residents of the holy city of Najaf.

Keywords: *Coagulase-negative Staphylococci, mecA gene, Multidrug-resistant, hemodialysis patients.*

Introduction

Coagulase-negative staphylococci (CoNS) are commensal bacteria of human skin and oral mucosa and have frequently detected as opportunistic pathogens[1], and regularly come upon in the hospital situation[2]. Susceptible patients accept the infection from the reservoirs[3]. Foreign bodies implantation and central nervous catheterization infections are mostly associated with CoNS[4]. Indwelling- devices carry and immunocompromised patients are more prone to these pathogens[5]. 46-65% of hospitalized patients carry CoNS [6,7].

Corresponding author
Oday Mitib Hadi
E. mail(s): kuh.aud@atu.edu.iq, ihsantag1975@gmail.com

Bacterial infections in the end stage of renal disease (hemodialysis) persons are the major cause of mortality and morbidity [8,9].

Coagulase-negative Staphylococci usually are much more resistant to antibiotics than *Staphylococcus aureus* [10]. Methicillin resistance (MR) has been widely studied in *Staphylococcus aureus* but slight is known about MR-CoNS [11].

Dissemination of antibiotic resistance genes among CoNS strains are predominant, and they are may be horizontally transferred from *S. aureus* [11], furthermore, MR gene is dramatically acquired by CoNS organisms [12]. MR in staphylococci is determined by the gaining of the *mecA* gene, that encodes PBP2A, a transpeptidase with a low attraction for β-lactams [13]. The treatment of *"S. epidermidis*" must be preceded by the determination of the gene responsible for methicillin resistance (*mecA*)

when it exhibits moderate susceptibility to Cefoxitin [14]. *mecA* is portion of the mec complex, which contains its repressor genes *mecI* and *mecR1* [15].

Repressor and inducer genes are responsible for the expression of *mecA* like the production of the enzyme β-lactamase [12], and all of the regulator, repressor and *mecA* genes lies on, dimensions and structure variable, mobile elements called Staphylococcal cassette chromosome mec (SCC mec) which was previously studied [16]. Thus, this study was conducted to investigate the prevalence of *mecA* gene in CoNS.

Materials and Methods

Samples Collection and bacterial strains identification

Staphylococci isolates obtained from hemodialysis patients who were admitted to the center of hemodialysis in Al-Sader Medical City, Najaf-Iraq" from July 2019 to January 2020. A total of 240 isolates were re-diagnosed in the laboratory by using traditional procedures, furthermore, vitek-2 system was used to establish the diagnosis.

Antimicrobial Susceptibility

Conventional Kirby- Bauer technique was used to detect the susceptibility of isolated bacterial strains to the antimicrobial agents (Oxoid ^{TM}) (i. e. Kanamycin (K) (30 µg/disc), Gentamycin (GM) (30 µg/disc), Fusidic acid (FD) (10 µg/disc), Rifampin (RD) (30 µg/disc), Tetracycline (TE) (30 µg/disc), Erythromycin (E) (30 µg/disc), and Tobramycin (TOB) (30 µg/disc). Clinical laboratory standard institute recommendations[17] were used as a comparative reference for comparing the zone of inhibition of antibiotics under test.

Bacterial DNA extraction

Bacterial DNA was extracted by using the wizard® genomic DNA purification kit protocol (Promega, USA).

Detection of *mec A* gene by PCR method

Primers and procedures previously used by [19,20] were used in this study. To detect the *mec A* gene (table 3-2), the DNA template was diluted 1 in 100. A 20 µL reaction mixture contained 2 µL of DNA template, 0.5 units of Taq polymerase (Thermo-fisher) and a final concentration of the following: 200 M of each dNTP (Pharmacia Biotech, St Albans, UK), 1.5 mM $MgCl_2$, 75 mM Tris–HCl pH 9.0, 20 mM $(NH4)_2SO4$, 0.01% Tween 20, 250 nM primers".

Thirty cycles of amplification were performed, each consisting of 60 s at 95°C, 45 s at 51°C and 2 min at 72°C. Ten microliters of PCR products were separated by electrophoresis on a 1% agarose gel and visualized under ultraviolet light after staining. with ethidium bromide. The target gene (*mecA*) sequence used in this study "Forward 5'-AAA ATC GAT GGT AAA GGT TGG C-3'" and Reverse primer was "R5'-AGT TCT GCA GTA CCG GAT TTG C-3'", with product size 532 base pairs [19,20].

Ethics Approval

A valid consent was achieved from each patient orally before their inclusion in the study.

Results

From July 2019 to January 2020, a total of 240 samples were collected randomly from the hemodialysis center, Al-Sader Medical City, Najaf-Iraq. The mean age of all patients was 52.4 years old. The age group >=60 years old occupied the highest percentage among others, (ρ>0.000). Other age groups take different ratios they were 16.7%, 27.9%, 16.7% and 9.6% for 20-29, 30-"39, 40-49 and 50-59 years old, respectively, table 1.

Table 2 illustrates the distribution of hemodialysis patients according to gender. Males significantly (ρ>0.000) higher than females and the male/female ratio was 2.

Table 1 Distribution of the infected patients according to the age groups

Age group	No. (Percent %)
20-29	40 (16.7%)
30-39	67 (27.9%)
40-49	40 (16.7%)
50-59	23 (9.6%)
>=60 years	70 (29.25%)
Total	240 (100.0%)

Table 2 Distribution of the infected patients according to the sex

		No. (Percent %)	Male/Female ratio
Sex	Male	160 (66.7%)	2
	Female	80 (33.3%)	
Total		240 (100.0)	

Our study results demonstrated that rural area has significantly low percent (40%) in comparison to an urban area (60%), table 3.

Staphylococcus epidermidis in our study (table 4) occupied the highest significantly (ρ> 0.000) percentage among other bacterial isolates. It was 63.33% followed by *S. aureus* (20%) and finally *S. saprophyticus* (16.67%). Out of this CoNS, only 46 (23.95%) isolates were identified to be methicillin-resistant i. e. *mecA* positive figure 1, of these 40 isolates (86.96%) *S. epidermidis* and 6 isolates (13.04%) *S. saprophyticus*.

Table 3 Distribution of the infected patients according to the residency

		No. (Percent %)
Residency	Rural	96 (40.0%)
	Urban	144 (60.0%)
	Total	240 (100.0%)

Table 4 The percentages of Staphylococci species isolation

	No. of isolates (%)	mec A gene#	
		Positive Strains	Negative Strains
S. aureus	48 (20%)	N.T.*	N.T.
S. epidermidis	152 (63.33%)	40 (26.32%)	112 (73.68%)
S. saprophyticus	40 (16.67%)	6 (15%)	34 (85%)

* N. T. = Not tested

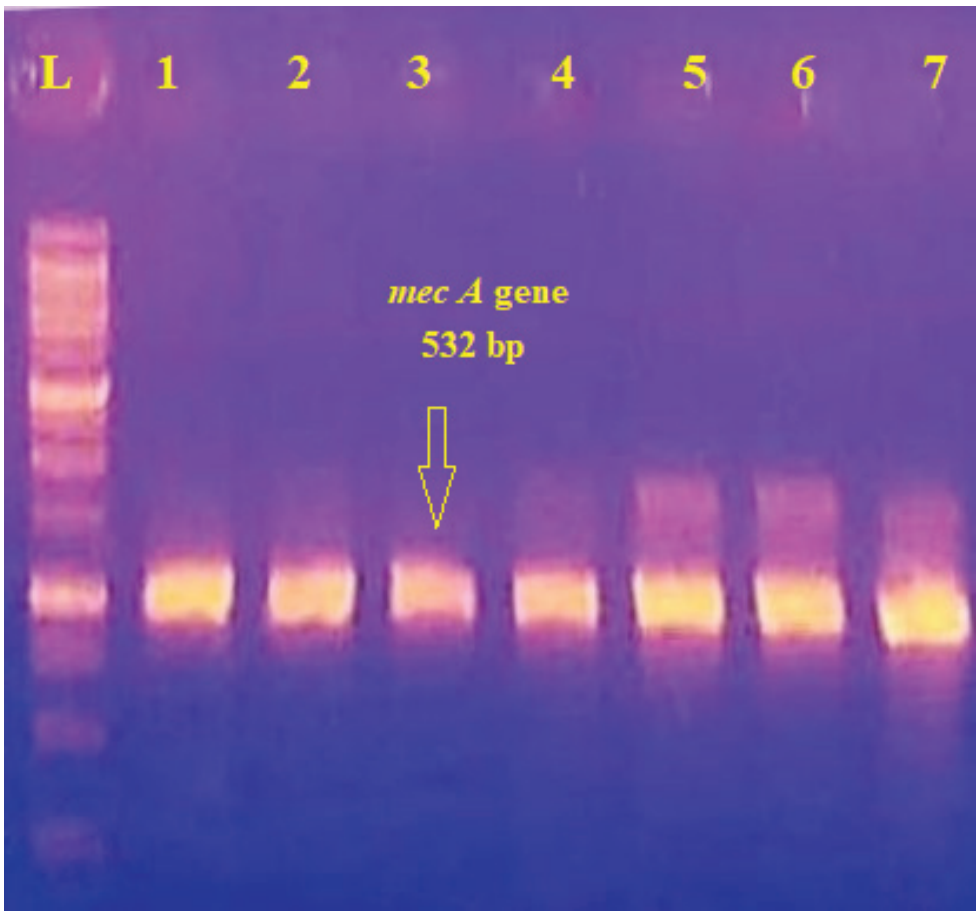

Figure 1 Ethidium bromide-stained gel electrophoresis products of *mec*A gene 532 bp.

Lane L= DNA ladder marker, Lane 1,2,3,4,5,6 and 7**.**

In table 5 all tested strains expressed phenotypic MR to non-beta-lactam antibiotics, as follows: 28 (60.87%) for Rifampin (RD), 27 (58.70%) for Gentamycin (GM) and Tobramycin (TOB), 26 (56.52%) for Fusidic acid (FD), 22 (47.83%) for Kanamycin (K) and Tetracycline (TE), and 21 (45.65%) for Erythromycin (VA).

Table 5 Antibiotic Susceptibility Patterns of all methicillin-resistant (MR), coagulase-negative staphylococcus (CoNS) isolates (n = 46) to all used antibiotics

	Number of tested isolates, Staphylococcus epidermidis 40 isolates, and Staphylococcus saprophyticus 6 isolates		
Antimicrobial agent (symbol)	**Sensitive No. (%)**	**Intermediate sensitivity No. (%)**	**Resistant No. (%)**
Kanamycin (K)	19 (41.30%)	5 (10.87%)	22 (47.83%)
Gentamycin (GM)	15 (32.61%)	4 (8.70%)	27 (58.70%)
Fusidic acid (FD)	17 (36.96%)	3 (6.52%)	26 (56.52%)
Rifampin (RD)	14 (30.43%)	4 (8.70%)	28 (60.87%)
Tetracycline (TE)	22 (47.83%)	2 (4.35%)	22 (47.83%)
Erythromycin (E)	20 (43.48%)	5 (10.87%)	21 (45.65%)
Tobramycin (TOB)	16 (34.78%)	3 (6.52%)	27 (58.70%)

Discussion

Present study results reveal that the infection among hemodialysis patients was in high percent (29.25%) in the age group more than 60 years old, and especially in males (66.7%) comparing with females. Also, our findings demonstrated that the urban area was in danger compared with rural areas, due to the increase ratio (60.0%) of infection in this place.

Recently, [20] published that the age range of the dialysis patients was 20-80 years old with a mean age of 47.9 years for all populations of the study. Also, they the higher ratio (34.21%) of the dialysis patients in the age group 51-60 years old. They were published that the ratio of male/ female was 2.8% (i. e. 28 male/ 10 female).

More men than women were on hemodialysis patients, they were 59% versus 41% [20]. The men and women age were 67.7 and 68.3 for men and women, respectively [21].

Previously, Maripuri[22] *et. al*. 2012 were reported that of 204,463 dialysis patients, 80% were urban; 10.2% micropolitan, and 9.8% rural, all those patients were detected to be 18 years old and older [22]. In Australia, they were significant differences between city and rural patients [23].

Different ratios of methicillin-resistant were reported previously, Ruppe' *et. al.* (2009), published that the percent of MR CoNS (*S. epidermidis* and *S. haemolyticus*) was 28.6%, 17.1%, 11.4% and 31.0% for Algeria, Mali, Moldova, and Cambodia, respectively [3]. Other research published that SCC *mec* typing of 274 carriage isolates of MR CoNS isolates from 154 adult WA Yampi Amerindians during the 2006 and 2008 were 89 (32.49%) *S. epidermidis*, 78 (28.47%) *S. haemolyticus*, 72 (26.28%) *S. hominis*, 27 (9.86%) *S. saprophyticus* and 8 (2.9%) other staphylococci [24]. *S. epidermidis* represent (69-84%) of the MR-CoNS [25]. *S. epidermidis* was the predominant species among others [26], also *S. epidermidis* was mentioned to be occupied

the highest percentage of both blood (19 "86.4%") and catheter (17"85%") [27]. Yamada[28] *et. al.* 2017 published that 55 (78.6%) of CoNS was *S. epidermidis* [28]. Also, Al-Janabi *et. al.* [20] found that 38 (100%) of *S. epidermidis* isolates carrying the *mecA* gene.

Antibiotic susceptibility patterns illustrated that the resistance ratio varied according to the type of antibiotics. These results mentioned that Rifampin (RD) has the highest resistant percent (60.87%), other antibiotics gave different ratios (table 5).

Previous study results reported that the phenotypic multidrug-resistant were detected in all CoNS strains, 57 (62%) were resistant to kanamycin, 38 (41%) to tobramycin and gentamicin, 44 (48%) to erythromycin, 45 (49%) to cotrimoxazole, 51 (55%) to tetracycline, 16 (17%) to rifampin, and 2 (2%) to Fosfomycin, also they found that the rate of MR-CoNS varies from one country to another [29].

Recently Al-Janabi *et. al.* [20] reported that there were different susceptibility patterns towards several antibiotics used in their study, they found that the highest rate (100%) of resistant to methicillin, penicillin, cefoxitin, ciprofloxacin clindamycin, and chloramphenicol. On the other hand, they found different ratios of resistance to other antibiotics.

From the previous results we concluded that there an increase in antibiotic-resistant by coagulase-negative staphylococci, espicially those straines which harboring *mec A* gene.

Acknowledgement: Great thanks to all doctors and health care workers for their advice to accomplish this research.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

1. Kloos WE, Bannerman TL. Update on the clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev. 1994; 7:117–40. https://doi.org/10.1128/cmr.7.1.117.

2. Jarvis WR, Martone WJ. Predominant pathogens in hospital infections. J Antimicrob Chemother. 1992;29 Suppl A:19–24. https://doi.org/10.1093/jac/29.suppl_a.19.

3. Ruppé E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, *et al*. Diversity of staphylococcal cassette chromosome mec structures in methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* strains among outpatients from four countries. Antimicrob Agents Chemother. 2009;53(2):442–9.

4. Sarnak MJ, Jaber BL. Mortality caused by sepsis in patients with end-stage renal disease compared with the general population. Kidney Int. 2000; 58:1758–64. https://doi.org/10.1111/j.1523-1755.2000.00337.x.

5. Aires De Sousa M, Santos Sanches I, Ferro ML, De Lencastre H. Epidemiological study of staphylococcal colonization and cross-infection in two West African Hospitals. Microb Drug Resist. 2000; 6:133–41.

6. Shobha KL, Rao PS, Thomas J. Survey of Staphylococcus isolates among hospital personnel, environment and their antibiogram with special emphasis on methicillin resistance. Indian J Med Microbiol. 2005; 23:186–8.

7. Wielders CL, Vriens MR, Brisse S, de Graaf-Miltenburg LA, Troelstra A, Fleer A, *et al.* In-vivo transfer of mecA DNA to *Staphylococcus aureus*. Lancet. 2001; 357:1674–5.

8. van Dijk PC, Jager KJ, de Charro F, et al. Renal replacement therapy in Europe: the results of a collaborative effort by the ERA-EDTA registry and six national or regional registries. Nephrol Dial Transplant. 2001; 16:1120–9.

9. Cimiotti JP, Wu F, Larson E. Emergence of resistant staphylococci on the hands of new graduate nurses. Infect Control Hosp Epidemiol. 2004; 25:431–5.

10. Huebner J, Goldmann DA. Coagulase-negative staphylococci: role as pathogens. Annu Rev Med. 1999; 50:223–36.

11. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the

SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis. 2001; 32 Suppl. 2: S114–32.

12. Hellmark B, Unemo M, Nilsdotter-augustinsson Å, And Söderquist B. Antibiotic susceptibility among *Staphylococcus epidermidis* isolated from prosthetic joint infections with special focus on rifampicin and variability of the *rpo*B gene. Clin Microbiol Infect. 2009;15(3):238–44.

13. Weller TM. The distribution of *mecA, mecR1* and *mecI* and sequence analysis of *mecI* and the *mec* promoter region in staphylococci expressing resistance to methicillin. J Antimicrob Chemother. 1999;43(1):15–22. https://doi.org/10.1093/jac/43.1.15.

14. Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, et al. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent *mec*A, *mec*I, *mec*R1, *bla*Z, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2011;55(8):3765–73.

15. Hecking M, Bieber BA, Ethier J, Kautzky-Willer A, Sunder-Plassmann G, Säemann MD, et al. Sex-specific differences in hemodialysis prevalence and practices and the male-to-female mortality rate: the Dialysis Outcomes and Practice Patterns Study (DOPPS). PLoS Med. 2014;11(10): e1001750. https://doi.org/10.1371/journal.pmed.1001750.

16. Fishovitz J, Hermoso JA, Chang M, Mobashery S. Penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. IUBMB Life. 2014; 66 (8):572–7. https://doi.org/10.1002/iub.1289.

17. Katayama Y, Ito T, Hiramatsu K. Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS431-mediated *mecI* deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus*. Antimicrob Agents Chemother. 2001; 45:1955–63.

18. Clinical and Laboratory Standard Institute (CLSI). (2018). Performance Standards for Antimicrobial Susceptibility Testing. 28th edition CLSI supplement M100. Wayne, PA.

19. de Silva GD, Kantzanou M, Justice A, Massey RC, Wilkinson AR, Day NP, et al. The *ica* operon and biofilm production in coagulase-negative Staphylococci associated with carriage and disease in a neonatal intensive care unit. J Clin Microbiol. 2002;40(2):382–8. https://doi.org/10.1128/jcm.40.02.382-388.2002.

20. Al-Janabi RA, Mohammed Ali AJ, Al-Saadi HA. Phenotypic and Genotypic Detection of Biofilm in Staphylococcus epidermidis isolated from some Clinical Specimens in Kerbala Province. Biochem Cell Arch. 2018;19(1):737–41.

21. Weigert A, Drozdz M, Silva F, Frazão J, Alsuwaida A, Krishnan M, et al. Influence of gender and age on haemodialysis practices: a European multicentre analysis. Clin Kidney J. 2019;13(2):217–24. https://doi.org/10.1093/ckj/sfz069.

22. Maripuri S, Arbogast P, Ikizler TA, Cavanaugh KL. Rural and micropolitan residence and mortality in patients on dialysis. Clin J Am Soc Nephrol. 2012;7(7):1121–9. https://doi.org/10.2215/CJN.10831011.

23. Gray NA, Dent H, McDonald SP. Renal replacement therapy in rural and urban Australia. Nephrol Dial Transplant. 2012;27(5):2069–76. https://doi.org/10.1093/ndt/gfr584.

24. Lebeaux D, Barbier F, Angebault C, Benmahdi L, Ruppé E, Felix B, et al. Evolution of nasal carriage of methicillin-resistant coagulase-negative staphylococci in a remote population. Antimicrob Agents Chemother. 2012;56(1):315–23. https://doi.org/10.1128/AAC.00547-11.

25. Silva FR, Mattos EM, Coimbra MV, Ferreira-Carvalho BT, Figueiredo AM. Isolation and molecular characterization of methicillin-resistant coagulase-negative staphylococci from nasal flora of healthy humans at three community institutions in Rio de Janeiro City. Epidemiol Infect. 2001;127(1):57–62. https://doi.org/10.1017/s095026880100574x.

26. Garza-González E, López D, Pezina C, Muruet W, Bocanegra-García V, Muñoz I, et al. Diversity of staphylococcal cassette chromosome mec structures in coagulase-negative staphylococci and relationship to drug resistance. J Med Microbiol. 2010;59(3):323–9.

27. Liakopoulos V, Petinaki E, Efthimiadi G, Klapsa D, Giannopoulou M, Dovas S, et al. Clonal

relatedness of methicillin-resistant coagulase-negative staphylococci in the haemodialysis unit of a single university centre in Greece. Nephrol Dial Transplant. 2008;23(8):2599–603. https://doi.org/10.1093/ndt/gfn101.

28. Yamada K, Namikawa H, Fujimoto H, Nakaie K, Takizawa E, Okada Y, et al. Clinical Characteristics of Methicillin-resistant Coagulase-negative Staphylococcal Bacteremia in a Tertiary Hospital. Intern Med. 2017;56(7):781 5. https://doi.org/10.2169/internalmedicine.56.7715.

29. Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle-Vavra S, Daum RS, Hiramatsu K. Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents Chemother. 2002 Apr;46(4):1147-52. https://doi:10.1128/aac.46.4.1147-1152.