ORIGINAL

Genotypic and phenotypic assessment of antibiotic resistance of MRSA bacteria isolated from food stuffs

Evaluación genotípica y fenotípica de la resistencia a los antibióticos de bacterias MRSA aisladas de alimentos

Seyyed Shahab Aldin Hosseini[1] ⓘ, Seyed Amirshayan Makkie[2] ⓘ, Bahareh Tavakoli-Far[3,4] ⓘ, Farhad Safarpoor Dehkordi[5] ⓘ

1. Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
2. Faculty of Science, Earth Sciences Building, 2207 Main Mall, Vancouver, British Columbia, Canada.
3. Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran.
4. Department of Physiology and Pharmacology, School pf Medicine, Alborz University of Medical Sciences, Karaj, Iran.
5. Halal Research Center of IRI, FDA, Tehran, Iran.

Corresponding author
Seyed Amirshayan Makkie
Faculty of Science, Earth Sciences Building,
2207 Main Mall, Vancouver, British Columbia, Canada
E-mail: shayaneo@gmail.com

Abstract

Background: Methicillin-Resistant *Staphylococcus aureus* (MRSA) bacteria are one of the chief causes of food-borne diseases. This survey was done to assess the genotypic and phenotypic profiles of antibiotic resistance of MRSA isolates of foodstuffs.

Methods: Forty MRSA strains isolated from foodstuffs were selected. Isolates were approved using the biochemical tests and also cefoxithin and oxacillin susceptibility testing. MRSA isolates were phenotypically assessed by the disk diffusion. DNA was extracted from the MRSAS isolates and subjected to PCR identification of antibiotic resistance genes.

Results: MRSA bacteria harbored the high prevalence of resistance toward penicillin (100%), tetracycline (95.00%), eryhtromicin (90.00%), and gentamicin (87.50%) antibiotics. *AacA-D* (55.00%), *ermA* (50.00%), and *tetK* (50.00%) had the highest distributions amongst examined antibiotic resistance genes.

Conclusion: Foodstuffs may be sources of resistant-MRSA, which pose a hygienic threat concerning their consumption. Nevertheless, further investigations are compulsory to understand additional epidemiological and molecular features of MRSA in foodstuffs.

Keywords: Methicillin-resistanmt *Staphylococcus aureus*, phenotype, genotype, antibiotic resistance, foodstuffs.

Resumen

Antecedentes: La bacteria Staphylococcus aureus resistente a la meticilina (SARM) es una de las principales causas de enfermedades transmitidas por los alimentos. Este estudio se realizó para evaluar los perfiles genotípicos y fenotípicos de la resistencia a los antibióticos de los aislados de SARM de los alimentos.

Métodos: Se seleccionaron 40 cepas de SARM aisladas de productos alimenticios. Los aislados se aprobaron mediante pruebas bioquímicas y también de susceptibilidad a la cefoxitina y la oxacilina. Los aislados de SARM se evaluaron fenotípicamente mediante la difusión en disco. Se extrajo el ADN de los aislados de SARM y se sometió a la identificación por PCR de los genes de resistencia a los antibióticos.

Resultados: Las bacterias MRSA presentaron una alta prevalencia de resistencia a los antibióticos penicilina (100%), tetraciclina (95,00%), eritromicina (90,00%) y gentamicina (87,50%). AacA-D (55,00%), ermA (50,00%) y tetK (50,00%) tuvieron las distribuciones más altas entre los genes de resistencia a los antibióticos examinados.

Conclusión: Los productos alimenticios pueden ser fuentes de SARM resistentes, lo que supone una amenaza higiénica en relación con su consumo. No obstante, es necesario realizar más investigaciones para conocer otras características epidemiológicas y moleculares del SARM en los alimentos.

Palabras clave: Staphylococcus aureus resistente a la meticilina, fenotipo, genotipo, resistencia a los antibióticos, productos alimenticios.

Introduction

Methicillin-Resistant Staphylococcus aureus (MRSA) is a a Gram-positive and catalase-positive bacterium and common phenomena of the S. aureus bacteria resistant to penicillins and cephalosporins[1]. The bacterium is responsible for severe cases of human clinical infections, including respiratory tract, urinary tract, blood, soft tissues, wound, burn and cerebrospinal infections with high distribution amongst hospithalized patients[2, 3]. It is responsible for about 100,000 infectious cases and 20-30% mortality in the United States[4]. MRSA bacteria are also substantial causes of food-borne diseases known with several clinical signs, particularly short incubation period and weakness vomiting, nausea, abdominal cramps, and toxic shock syndrome[5, 6].

MRSA bacteria caused more complicated diseases for a more extended period compared to other bacteria. Most of their isolates harboured complete resistance toward diverse classes of antibiotic agents, particularly fluoro-quinolones, aminoglycosides, tetracyclines, macrolides, cephalosporins, penicillins, and carbapenems[7]. Genetically, diverse antibiotic resistance genes are mainly responsible for the occurrence of antibiotic resistance in MRSA bacteria. TetK, ermA, gyrA, dfrA, rpoB, and aacA-D were considered as the most important antibiotic resistance genes in the MRSA isolates[8].

Rendering the high MRSA importance as a food-borne pathogen, the present survey was performed to assess the genotypic and phenotypic patterns of resistance of MRSA bacteria isolated from numerous ready-to-eat food samples.

Materials and methods

MRSA isolates
The present research was performed according to the Canadian Guidelines of Research Ethics in Medical Sciences. Through the summer of 2020, 40 MRSA isolates of numerous food samples were selected for this survey. Isolates were identified using Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase test, coagulase test (rabbit plasma), oxidase test, OF glucose test, bacitracin sensitivity test (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, Voges-Proskauer (VP) (Merck, Germany) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests[9]. MRSA identification was performed using cefoxitin and oxacillin susceptibility testing[10].

Phenotypically survey of antibiotic resistance
Mueller–Hinton agar (Merck, Germany) and principles of the Clinical Laboratory Standard Institute (CLSI) were applied for this purpose[11]. Phenotypic resistance of MRSA bacteria was assessed toward gentamicin (10 μg/disk), ciprofloxacin (5 μg/disk), clindamycin (2 μg/disk), erythromycin (15 μg/disk), penicillin (10 μg/disk), and tetracycline (30 μg/disk) (Oxoid, UK)[12, 13].

Genotypically survey of antibiotic resistance
DNA was extracted from MRSA isolates of food samples using DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany)[14-16]. Quality and quantity of extracted DNA were then checked[17-19]. Antibiotic resistance genes were detected using the Polymerase Chain Reaction (PCR) (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). **Table I** shows the PCR circumstances for antibiotic resistance genes detectionm[20-22]. Electrophoresis was done according to standard procedure[23, 24].

Results

All 40 MRSA isolates were assessed by the disk diffusion and PCR for phenotypicall and genotypicall assessment of antibiotic resistance, respectively. **Table II** shows the phenotypic resistance pattern of MRSA isolates. Disk diffusion showed that MRSA bacteria harbored the high prevalence of resistance toward penicillin (100%), tetracycline (95.00%), eryhtromicin (90.00%), and gentamicin (87.50%) antibiotics. The lowest resistance rate was obtained against ciprofloxacin (62.50%) and clindamycin (50.00%).

Table III shows the genotypic resistance pattern of MRSA isolates. *AacA-D* (55.00%), **ermA** (50.00%), and *tetK* (50.00%) had the highest distributions. However, *gyrA* (25.00%) and *rpoB* (30.00%) had the lowest distribution.

Table IV shows the simultaneous presence of antibiotic resistance encoding genes amongst the examined MRSA isolates. Majority of isolates harboured *aacA-D + ermA* (25.00%), *aacA-D + tetK* (25.00%), and *aacA-D + rpoB* (25.00%) antibiotic resistance encoding genes.

Discussion

MRSA is a nosocomial pathogen with both healthcare and community sources.It is also considered as a foodborne pathogens responsible for severe morbidity globally[25]. Contaminated food stuffs, especially those with animal origin, are determined as one of the likely causes of MRSA transmission to human[26]. Thus, MRSA in food stuffs has two paramount importance: food-borne diseases and transmission of antibiotic resistance.

The present survey was addressed the MRSA genotypic and phenotypic patterns of antibiotic resistance. MRSA

isolates of foodstuffs harboured the maximum resistance rate against penicillin (100%), tetracycline (95.00%), eryhtromicin (90.00%), and gentamicin (87.50%). Additionally, they harboured *aacA-D* (55.00%), *ermA* (50.00%), and *tetK* (50.00%) antibiotic resistance genes. Thus, phenotypic pattern of resistance was approved by the genotypic pattern. Iregular and indiscriminate antibiotic prescription are the main reasons for the high prevalence of resuistance amid the MRSAisolates. Similarly, high MRSA resistance rate toward penicillin, tetracycline, eryhtromicin, and gentamicin was also reported previously. Safarpoor Dehkordi et al. (2017)[27] reported that the *S. aureus* resistance rate toward penicillin, ceftaroline, gentamicin, amikacin, kanamycin, azithromycin, erythromycin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol and rifampin antibiotic agents were 100%, 10%, 81.08%, 70.27%, 43.24%, 59.45%, 86.48%, 100%, 81.08%, 48.64%, 43.24%, 48.64%, 83.78%, 29.72%, and 35.13%, respectively. They also showed that the distribution of *aacA-D, tetK, tetM, msrA, ermA, ermC,* and *linA* antibiotic resistance genes were 62.16%, 72.97%, 27.02%, 64.86%, 72.97%, 27.02%, and 43.24%, respectively. Findings of the present survey and those of Safarpoor Dehkordi et al. (2017)[27] were similar to those of Fowoyo and Ogunbanwo (2017)[28] and Akanbi et al.

(2017)[29]. High distribution of antibiotic resistance genes was also reported in surveys conducted in Algeria[30], China[31], and Egypt[32].

Rendering our results, only some of the MRSA bacteria, which harboured resistance to a particular group or a specific antibiotic carried the specific antibiotic resistance encoding gene to that particular antibiotic agent. This matter also found for other antibiotic agents and resistance genes. This finding is maybe because the presence of antibiotic resistance genes is only one of the known procedures for the occurrence of antibiotic resistance in bacteria[33-35].

Conclusion

In conclusion, MRSA isolates of foodstuffs harboured high prevalence of resistance and distribution of antibiotic resistance genes. This finding may show the role of food stuffs in the transmission of MRSA bacteria to human populations. Using high-quality raw materials, proper hygienic circumstances in food processing, adequate cooking of food samples, cross-contamination prevention, and antibiotic prescription rendering the outcomes of disk diffusion can diminish the risk of MRSA.

Table I: Primers and following conditions of the PCR[20].

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 °C ------------ 5 min. 25 cycle: 94 °C ------------ 60 s 55 °C ------------ 70 s 72 °C ------------ 60 s 1 cycle: 72 °C ------------ 10 min	5 µL PCR buffer 10X 1.5 mM Mgcl₂ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas) 2.5 µL DNA template
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
gyrA	F: AATGAACAAGGTATGACACC R: TACGCGCTTCAGTATAACGC	223	1 cycle: 94 °C ------------ 10 min. 25 cycle: 94 °C ------------ 20 s 52 °C ------------ 20 s 72 °C ------------ 50 s 1 cycle: 72 °C ------------ 5 min	5 µL PCR buffer 10X 2 mM Mgcl₂ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
dfrA	F: CTCACGATAAACAAAGAGTCA R: CAATCATTGCTTCGTATAACG	201	1 cycle: 94 °C ------------ 2 min. 30 cycle: 94 °C ------------ 60 s 50 °C ------------ 60 s 72 °C ------------ 60 s 1 cycle: 72 °C ------------ 5 min	5 µL PCR buffer 10X 2 mM Mgcl₂ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
rpoB	F: ACCGTCGTTTACGTTCTGTA R: TCAGTGATAGCATGTGTATC	460	1 cycle: 94 °C ------------ 5 min. 32 cycle: 94 °C ------------ 60 s 56 °C ------------ 45 s 72 °C ------------ 60 s 1 cycle: 72 °C ------------ 10 min	5 µL PCR buffer 10X 2 mM Mgcl₂ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template

Table II: Phenotypic resistance pattern of MRSA isolates.

N. MRSA Isolates	N (%) isolates resistant to each antibiotic					
	P10*	Gen	Ert	Tet	Cip	Clin
Food stuffs (40)	40 (100)	35 (87.50)	36 (90.00)	38 (95.00)	25 (62.50)	20 (50.00)

*P10: penicillin (10 µg/disk), Gen: gentamicin (10 µg/disk), Ert: erythromycin (15 µg/disk), Tet: tetracycline (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Clin: clindamycin (2 µg/disk).

Table III: Genotypic resistance pattern of MRSA isolates.

N. MRSA Isolates	N (%) isolates harbored each antibiotic resistance genes					
	aacA-D	ermA	tetK	gyrA	dfrA	rpoB
Food stuffs (40)	22 (55.00)	20 (50.00)	20 (50.00)	10 (25.00)	15 (37.50)	12 (30.00)

Table IV: Simultaneous presence of antibiotic resistance encoding genes amongst the examined MRSA isolates.

N. MRSA Isolates	N (%) isolates harbored each antibiotic resistance genes										
Food stuffs (40)	aacA-D + ermA	aacA-D + tetK	aacA-D + gyrA	aacA-D + dfrA1	aacA-D + rpoB	ermA + tetK	ermA + gyrA	ermA + dfrA1	ermA + rpoB	tetK + gyrA	tetK + dfrA1
	10 (25.00)	10 (25.00)	5 (12.50)	8 (20.00)	6 (15.00)	10 (25.00)	5 (12.50)	7 (17.50)	6 (15.00)	6 (15.00)	8 (20.00)

References

1. Madahi H, Rostami F, Rahimi E, Dehkordi FS. Prevalence of enterotoxigenic Staphylococcus aureus isolated from chicken nugget in Iran. Jundishapur journal of microbiology. 2014 Aug;7(8).

2. Daum RS. Skin and soft-tissue infections caused by methicillin-resistant Staphylococcus aureus. New England Journal of Medicine. 2007 Jul 26;357(4):380-90.

3. Khalili H, Sisakhtpour R, Abdollahzadeh S. Identification of biofilm producing bacterial species causing dental caries and their sensitivity to zoocin A and clove hydroalcoholic extract. Immunopathol Persa. 2018;4(2):e27.

4. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. Jama. 2007;298:1763-71.

5. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A, Momeni M. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. Journal of Applied Poultry Research. 2013 Dec 1;22(4):913-21.

6. Afhami S, Ali Borumand M, Esmailpour Bazzaz N, Saffar H, Hadadi A, Jafary Nezhad M, et al. Antimicrobial resistance pattern of Acinetobacter; a multicenter study, comparing European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI); evaluation of susceptibility testing methods for polymyxin. Immunopathol Persa. 2021;7(1):e04.

7. Hasanpour Dehkordi A, Khaji L, Sakhaei Shahreza MH, Mashak Z, Safarpoor Dehkordi F, Safaee Y, Hosseinzadeh A, Alavi I, Ghasemi E, Rabiei-Faradonbeh M. One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus recovered from raw meat. Trop Biomed. 2017;34(2):396-404.

8. Abdolmaleki Z, Mashak Z, Dehkordi FS. Phenotypic and genotypic characterization of antibiotic resistance in the methicillin-resistant Staphylococcus aureus strains isolated from hospital cockroaches. Antimicrobial Resistance & Infection Control. 2019 Dec;8(1):1-4.

9. Abdolmaleki Z, Mashak Z, Safarpoor Dehkordi F. Molecular and virulence characteristics of methicillin-resistant Staphylococcus aureus bacteria recovered from hospital cockroaches. Jundishapur Journal of Microbiology. 2019 Dec 31;12(12).

10. Rahi A, Kazemeini H, Jafariaskari S, Seif A, Hosseini S, Dehkordi FS. Genotypic and Phenotypic-Based Assessment of Antibiotic Resistance and Profile of Staphylococcal Cassette Chromosome mec in the Methicillin-Resistant Staphylococcus aureus Recovered from Raw Milk. Infection and drug resistance. 2020;13:273.

11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25. 2015.

12. Momeni Shahraki M, Shakerian A, Rahimi E, Safarpoor Dehkordi F. Study the frequency of enterotoxin encoding genes and antibiotic resistance pattern of methicillin-resistant Staphylococcus aureus isolated from vegetable and salad samples in Chaharmahal Va Bakhtiari province. Journal of Food Microbiology. 2020 Jun 21;7(2):55-69.

13. Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2019 Apr;39(2):e12594.

14. Dehkordi FS. Prevalence study of Bovine viral diarrhea virus by evaluation of antigen capture ELISA and RT-PCR assay in Bovine, Ovine, Caprine, Buffalo and Camel aborted fetuses in Iran. AMB express. 2011 Dec;1(1):1-6.

15. Dehkordi FS, Saberian S, Momtaz H. Detection and segregation of Brucella abortus and Brucella melitensis in Aborted Bovine, Ovine, Caprine, Buffaloes and Camelid Fetuses by application of conventional and real-time polymerase chain reaction. The Thai Journal of Veterinary Medicine. 2012 Mar 1;42(1):13.

16. Ranjbar R, Seif A, Safarpoor Dehkordi F. Prevalence of antibiotic resistance and distribution of virulence factors in the shiga toxigenic Escherichia coli recovered from hospital food. Jundishapur Journal of Microbiology. 2019 May 31;12(5).

17. Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences. 2011.

18. Ranjbar R, Farsani FY, Dehkordi FS. Phenotypic analysis of antibiotic resistance and genotypic study of the vacA, cagA, iceA, oipA and babA genotypes of the Helicobacter pylori strains isolated from raw milk. Antimicrobial Resistance & Infection Control. 2018 Dec;7(1):1-4.

19. Dehkordi FS, Momtaz H, Doosti A. Application of Real-Time PCR for detection of Aspergillus species in aborted ruminant foetuses. Bulgarian J. Vet. Med. 2012 Mar 1;15(1):30-6.

20. Momtaz H, Hafezi L. Meticillin-resistant Staphylococcus aureus isolated from Iranian hospitals: virulence factors and antibiotic resistance properties. Bosnian journal of basic medical sciences. 2014 Nov;14(4):219.

21. Mashak Z, Jafariaskari S, Alavi I, Shahreza MS, Dehkordi FS. Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat. Infection and drug resistance. 2020;13:257.

22. Ranjbar R, Dehkordi FS, Heiat M. The frequency of resistance genes in Salmonella enteritidis strains isolated from cattle. Iranian Journal of Public Health. 2020 May;49(5):968.

23. Dehkordi FS, Khamesipour F, Momeni M. Brucella abortus and Brucella melitensis in Iranian bovine and buffalo semen samples: The first clinical trial on seasonal, Senile and geographical distribution using culture, conventional and real-time polymerase chain reaction assays. Kafkas Univ Vet Fak Dergisi. 2014;20(6):821-.

24. Dehkordi FS, Valizadeh Y, Birgani TA, Dehkordi KG. Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immuno sorbent assay and duplex polymerase chain reaction. J Pure Appl Microbiol. 2014;8(2):1065-9.

25. Sergelidis D, Angelidis AS. Methicillin-resistant Staphylococcus aureus: a controversial food-borne pathogen. Letters in Applied Microbiology. 2017 Jun;64(6):409-18.

26. Fetsch A, Johler S. Staphylococcus aureus as a foodborne pathogen. Current Clinical Microbiology Reports. 2018 Jun;5(2):88-96.

27. Safarpoor Dehkordi F, Gandomi H, Akhondzadeh Basti A, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017;6:104.

28. Fowoyo P, Ogunbanwo S. Antimicrobial resistance in coagulase-negative staphylococci from Nigerian traditional fermented foods. Annals of clinical microbiology and antimicrobials. 2017;16:4.

29. Akanbi OE, Njom HA, Fri J, Otigbu AC, Clarke AM. Antimicrobial Susceptibility of Staphylococcus aureus Isolated from Recreational Waters and Beach Sand in Eastern Cape Province of South Africa. International journal of environmental research and public health. 2017;14:1001.

30. Achek R, Hotzel H, Cantekin Z, Nabi I, Hamdi TM, Neubauer H, El-Adawy H. Emerging of antimicrobial resistance in staphylococci isolated from clinical and food samples in Algeria. BMC research notes. 2018;11:663.

31. Li S-M, Zhou Y-F, Li L, Fang L-X, Duan J-H, Liu F-R, Liang H-Q, Wu Y-T, Gu W-Q, Liao X-P. Characterization of the Multi-Drug Resistance Gene cfr in Methicillin-Resistant Staphylococcus aureus (MRSA) Strains Isolated From Animals and Humans in China. Frontiers in microbiology. 2018;9:2925.

32. Osman K, Badr J, Al-Maary KS, Moussa IM, Hessain AM, Girah Z, Amin M, Abo-shama UH, Orabi A, Saad A. Prevalence of the antibiotic resistance genes in coagulase-positive-and negative-staphylococcus in chicken meat retailed to consumers. Frontiers in microbiology. 2016;7:1846.

33. Bolte J, Zhang Y, Wente N, Mahmmod YS, Svennesen L, Krömker V. Comparison of phenotypic and genotypic antimicrobial resistance patterns associated with Staphylococcus aureus mastitis in German and Danish dairy cows. Journal of dairy science. 2020 Apr 1;103(4):3554-64.

34. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS microbiology. 2018;4(3):482.

35. Hafizi M, Rajaei-Isfahani M, Noorian K. The effect of daily change of sterile urinary bag on bacteriuria and catheter–associated urinary tract infections. J Renal Inj Prev. 2018;7(4):240-5.