Detection of vancomycin resistance in multidrug-resistant Enterococcus faecalis isolated from burn infections

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Detection of vancomycin resistance in multidrug-resistant *Enterococcus faecalis* isolated from burn infections

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ABSTRACT

Background: *Enterococcus faecalis* is one of the main causes of nosocomial infections. The clinical importance of *E. faecalis* is related to its antibiotic resistance because it is intrinsic resistance. Vancomycin-resistant *E. faecalis* (VRE) acquired the vancomycin (VA) resistance by a plasmid that permits the sensitive bacteria to become resistant and has the ability to transfer this resistance to other unrelated bacteria. **Materials and Methods:** During the period of 3 months from 1st March to 31st May 2019, a total of 50 swabs were collected from patients with burn infections from patients in Al-Hilla General Teaching Hospital, Al-Hilla City, Babylon, Iraq. The swabs were cultured on selective and differential media and incubated aerobically at 37°C for 24–48 h. Using VITEK 2 system was used for more confirmative diagnosis. **Results:** All samples yielded bacterial growth 100%. A total of 71 bacterial isolates were identified. Morphological and biochemical characterization of bacterial cultures revealed that 14/71 (19.7%) of total isolates were *E. faecalis*. The disc diffusion method was used for antibacterial susceptibility test against 22 antibiotics. Among 14 isolates of *E. faecalis*, only 5 isolates (35.7%) have resistance against VA. **Conclusion:** The emergence of VRE became a great challenge with serious complications of the control policy for nosocomial infections in our hospitals.

KEY WORDS: Burn, *Enterococcus faecalis*, Iraq, Vancomycin resistance

INTRODUCTION

Enterococcus faecalis is Gram-positive cocci arranged in pairs or chains, it is a part of the normal flora of human's intestines. It is a facultative anaerobe.[1] *E. faecalis* is one of the main causes of nosocomial infections which usually occur with abdominal surgery, penetrating trauma, catheterization, and intravenous therapy such as urinary tract infections, endocarditis, bacteremia, meningitis, and surgery infections.[2] Due to its characteristic cell wall carbohydrate, *E. faecalis* was first identified as Group D streptococci till 1984 that it was classified as a separate genus called *Enterococcus*. The clinical importance of *E. faecalis* is related to its antibiotic resistance because it is intrinsic resistance.[3] Despite that the first identification of vancomycin-resistant *E. faecalis* (VRE) in the 1980s at Europe and United State only, VRE have been reported worldwide at the last decades. Nowadays, VRE have been reported worldwide.[4] Vancomycin (VA) is a member of the glycopeptides group. It acts as inhibitor of cell wall synthesis.[5] *Amycolatopsis orientalis* produces the VA. At first, the VA was used to treat methicillin-resistant *Staphylococcus aureus* (MRSA) which could not develop a significant resistance against it.[6] VA never became the first-line treatment due to its poor oral bioavailability and its toxicity (for the kidney and the ear).[7] VRE acquired the VA resistance by a plasmid and able for transferring of the VA resistance and permits the sensitive bacteria to become resistant.[8] The enterococci have six different types of VA resistance included Van-A, Van-B, Van-C, Van-D, Van-E, and Van-G. The highest resistance was shown by Van-A which has the ability to transfer the resistance to MRSA, while the lowest resistance was shown by Van-C.[9] VRE have caused many nosocomial infection outbreaks worldwide and Van A gene. The mechanism of VRE resistance to VA is alteration of synthesis pathway of the peptidoglycan.[10-12] The study of the genome sequence for *Enterococcus* was very necessary due to its many health dangers. *E. faecalis* has different metabolic strategies and has a wide range of regulatory systems.[1] The aim of the present study is the detection of VA resistance in the isolates of multidrug-resistant *E. faecalis* isolated from burn infection.

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MATERIALS AND METHODS

Collection of Samples

During the period of 3 months from 1st March to 31st May 2019, a total of 50 swabs were collected from patients with burn infections in Al-Hilla General Teaching Hospital, Al-Hilla City, Babylon, Iraq. The swabs were cultured on selective and differential media and incubated aerobically at 37°C for 24–48 h. According to their features, the bacterial isolates were diagnosed by the comparison with MacFaddin.[13] Using VITEK 2 system was used for more confirmative diagnosis.

Antibacterial Susceptibility Test

The disc diffusion test was performed for 22 antibiotic discs in HiMedia/India. According to Bauer *et al*.[14] method, bacterial suspension was prepared by adding five young colonies of *E. faecalis* to 5 ml of brain heart infusion broth and incubated at 37°C for 18–24 h. After incubation, standard McFarland tube was prepared. An inoculum was obtained from the bacterial suspension and streaked on the Mueller-Hinton agar. The antibiotic disc was placed on the surface of Mueller-Hinton agar by a flamed forceps. Overnight incubation at 37°C for 18–24 h. The diameter of inhibition zone was measured by a ruler and compared to standard criteria in CLSI.[15]

RESULTS AND DISCUSSION

During the period of 3 months from 1st March to 31st May 2019, a total of 50 swabs were collected from patients with burn infections from patients in Al-Hilla General Teaching Hospital, Al-Hilla City, Babylon, Iraq. All samples yielded bacterial growth 50 (100%). A total of 71 bacterial isolates were identified. This finding refers to the positive culture with a mixture of bacterial isolates which were considerable.[16] The laboratory diagnostic of bacterial isolates was compared with the standard features in authorized global references, for example, Collee *et al*.[17] and MacFaddin.[13] The characteristics being investigated for diagnosis are colonial and cellular morphology on selective and differential media and biochemical tests, Table 1.

Table 1: Diagnostic characteristics of *Enterococcus faecalis* in the present study

Number	The test	The result
1	Gram stain	Gram-positive cocci
2	Cells arrangement	Diplococci or short chains
3	Hemolysis	Non-hemolytic
4	Lancefield group	Group D
5	Motility	Non-motile
6	Spore forming	Non-spore forming
7	Oxidase	Negative
8	Catalase	Negative
9	Growth on MacConkey agar	Positive
10	Tolerance of bile salt	Positive

In our study, among 71 bacterial isolates, *E. faecalis* represented 14/71 (19.7%) according to the morphological and biochemical characterization and Vitek 2 system, Figures 1 and 2. The isolation rate of *E. faecalis* in the other studies was a variable Garges *et al.,*[18] 10%; Trotman and Bell,[19] 15%; Cohen-Wolkowiez *et al*.,[20] 6.6%; Alfaleh *et al*.,[21] 2.04%; and Youssef *et al*.,[22] 16.6%. In Brazil, Strabelli *et al*.[23] documented that *E. faecalis* caused an outbreak of bacteremia. The outbreaks by endemic *E. faecalis* infections are an indication of horizontal transmission due to the immunosuppression conditions.[24]

Despite *E. faecalis* is a part of normal flora in the human, in the 1980s, it was reported as an important nosocomial pathogen with high mortality due to its various survival and virulence factors.[25,26] It is ability for competition with other bacteria, represents one of the survival factors of *E. faecalis*; in addition to, it is

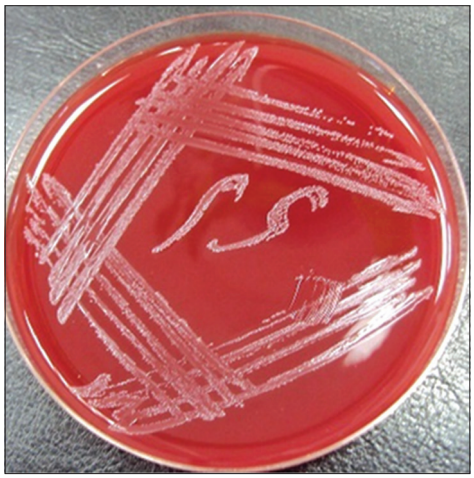

Figure 1: *Enterococcus faecalis* on blood agar

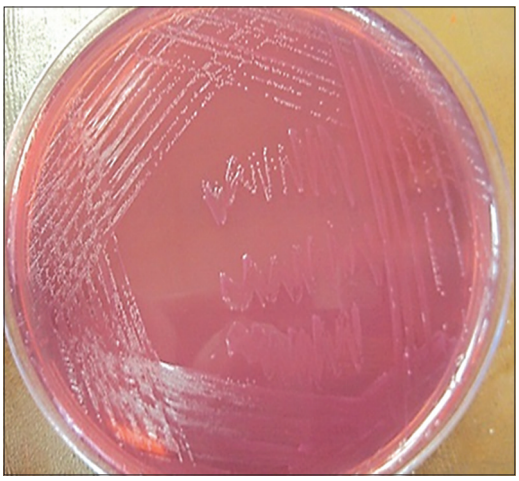

Figure 2: *Enterococcus faecalis* on MacConkey agar

tolerance of hard nutritional conditions and very harsh environmental conditions such as ethanol, detergents, pH (9.6), azide, extreme concentrations of salts, bile salts tolerance, heavy metals, and desiccation tolerance,[1] in addition to the ability to survive for long periods on inanimate objects such as stethoscopes and thermometers and the growth in wide range of the temperature (10–45)°C, and in addition to, it is ability to tolerance the very high temperatures of 60°C for 30 min.[26,27] *E. faecalis* has many virulence factors including biofilm, lytic enzymes, suppression of immunity system, and cytolysin.[28,29] In addition, the high levels of antibiotic resistance contribute to *E. faecalis* pathogenicity, especially since VA-resistant *E. faecalis* is becoming most common.[30,31]

As detailed in Tables 2 and 3, full resistance 100% was shown in each ampicillin, amoxicillin/clavulanic acid, Cephalothin (CEF), cefotaxime, and ceftazidime, and high levels of resistance (85.7%) against each cost per mile and click-through rate, and 78.6% against oxacillin, Tables 2 and 3. These are β-lactam antibiotics that act as inhibitor of bacterial cell wall biosynthesis. These high resistance levels may ascribable to the frequently use β-lactam antibiotics by patients which due to randomly use of these antibiotics. The resistance against β-lactams usually results from β-lactamases, efflux pumps, and modification of penicillin-binding protein.[32,33]

In the present study, fully sensitivity (100%) was reported against each of imipenem (IPM) and meropenem (MRP) for all isolates [Tables 2 and 3]. This very high sensitivity can be attributed to the fact that carbapenems (IPM and MRP) are the effective antibiotics because they are broad-spectrum antibiotics, and it has β-lactam ring that has resistance to hydrolysis by most β-lactamases.[34] This result was in accordance with.[35,36]

On the other hand, low levels of resistance (14.3%) were shown against ciprofloxacin (CIP), norfloxacin (NX), and Clindamycin (DA). These findings are in agreement with Mitscher[37] who has stated that fluoroquinolones (NX and CIP) are effective antibiotics which can inhibit bacterial growth by effecting DNA maintenance; therefore, many types of Gram-positive bacteria were sensitive to it. On the other hand, our result about DA was in agreement with Al-Hassnawi[38] who documented low levels of resistance (11.3%) against DA, but in a variability with other studies: Hussain *et al*.,[39] 23% and Sattler *et al*.,[40] 35%. It is noteworthy that many reasons may lead to the variations in the resistance levels among the different studies such as follows: The virulence factors that found in some isolates and absent in others, the source of isolate, the conditions of test, and the type of technique (Brown *et al.,* 2005).[41]

In this study, the resistance of *E. faecalis* against aminoglycoside antibiotics such as tobramycin,

Table 2: The percentage of antibiotic resistance in *Enterococcus faecalis* isolates

Antibiotic	AK	AMC	AMP	CPM	CTX	CAZ	CTR	KF	C	CIP	DA	GEN	IPM	K	MRP	M	NX	OX	RA	TE	TOB	VA
Concentration mcg*	30	30	10	30	30	30	30	30	10	5	10	10	10	30	10	5	10	1	5	10	10	30
Resistance (%)	50	100	100	85.7	100	100	85.7	100	42.9	14.3	14.3	64.3	0	64.3	0	64.3	14.3	78.6	42.9	64.3	64.3	35.7

*According to the manufacturing company HiMedia/India. AK: Amikacin, AMC: Amoxiclav, AMP: Ampicillin, CPM: Cefepime, CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, CEF: Cephalothin, C: Chloramphenicol, CIP: Ciprofloxacin, DA: Clindamycin, GEN: Gentamicin, IPM: Imipenem, K: Kanamycin, MRP: Meropenem, M: Methicillin, NX: Norfloxacin, OX: Oxacillin, RA: Rifampicin, TE: Tetracyclin, TOB: Tobramycin, VA: Vancomycin

Table 3: Antibiotic resistance in 14 isolates of *Enterococcus faecalis* in the present study

Number	Antibiotic	Number of isolates													
1	AK	S	R	R	R	S	S	S	R	S	R	S	R	S	R
2	AMC	R	R	R	R	R	R	R	R	R	R	R	R	R	R
3	AMP	R	R	R	R	R	R	R	R	R	R	R	R	R	R
4	CPM	R	R	R	R	R	R	R	R	R	R	S	R	R	S
5	CTX	R	R	R	R	R	R	R	R	R	R	R	R	R	R
6	CAZ	R	R	R	R	R	R	R	R	R	R	R	R	R	R
7	CTR	R	R	R	S	R	R	R	R	S	R	R	R	R	R
8	KF	R	R	R	R	R	R	R	R	R	R	R	R	R	R
9	C	R	S	R	R	S	S	S	R	R	R	S	S	S	S
10	CIP	S	R	R	S	S	S	S	S	S	S	S	S	S	S
11	DA	R	R	S	S	S	S	S	S	S	S	S	S	S	S
12	GEN	R	R	R	S	S	R	R	R	R	S	S	R	S	R
13	IPM	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14	K	R	R	R	S	R	S	S	R	R	R	R	R	S	S
15	MRP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
16	M	R	R	R	S	S	S	R	R	R	R	S	R	R	S
17	NX	S	R	R	S	S	S	S	S	S	S	S	S	S	S
18	OX	R	R	R	R	S	S	R	R	R	R	R	R	S	R
19	RIF	R	R	R	S	S	S	R	S	S	R	R	S	S	S
20	TE	R	R	R	S	S	R	R	R	R	R	S	S	R	S
21	TOB	R	R	R	R	R	S	S	R	R	R	S	S	S	R
22	VA	R	R	R	R	S	S	S	S	S	S	S	S	S	R

AK: Amikacin, AMC: Amoxiclav, AMP: Ampicillin, CPM: Cefepime, CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, KF: Cephalothin, C: Chloramphenicol, CIP: Ciprofloxacin, DA: Clindamycin, GEN: Gentamicin, IPM: Imipenem, K: Kanamycin, MRP: Meropenem, M: Methicillin, NX: Norfloxacin, OX: Oxacillin, TE: Tetracycline, TOB: Tobramycin, VA: Vancomycin

Figure 3: Multidrug-resistant *Enterococcus faecalis*

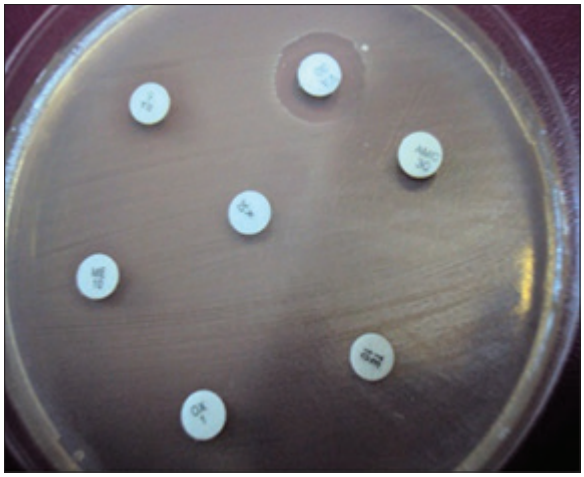

Figure 4: Vancomycin-resistant *Enterococcus faecalis*

gentamicin, amikacin, and kanamycin was ranged from 50% to 64.3%, Table 2. This is in agreement with the results by Panesso *et al*.[42]

As detailed in Table 2, *E. faecalis* has low resistance against rifampicin (42.9%) and chloramphenicol (42.9%). The low resistance to rifampin can be ascribable to the fact that rifampin resistance needs long period of time to develop, and rifampin could not act individually as antibacterial therapy but by the synergistic with other antibiotics to decrease the resistance.[15,43,44] It is noteworthy that *E. faecalis* has resistance against tetracycline (64.3%) and methicillin (64.3%), Table 2.

In this study, all evidences were indicating that *E. faecalis* has a multidrug resistance, Figure 3. Its intrinsic resistance and its ability to acquire the resistance genes made *E. faecalis* become a serious part of nosocomial pathogens. Recently, this bacteria developed resistance against many different groups of antibiotics.

As detailed in Tables 2 and 3, five isolates of *E. faecalis* have resistance against VA 35.7%, Figure 4. VA is a glycopeptide antibiotic. Because it is a toxic agent for the kidney as well as it is intravenously used, VA represented the last line for the treatment of the Gram-positive bacterial infections.[45] Furthermore, VA is intravenously used, so the adaptation of bacteria will be slow against it, which kept VA alive for years and had reduced the bacterial opportunities to develop the resistance.[46,47] The emergence of VRE is a dangerous nosocomial problem with serious complications of the control policy for nosocomial infections. In a study of teaching hospitals in France, glycopeptide-resistant

enterococci isolated from the hospitalized patients. This resistance may ascribable to the increasing use of VA as the last resort treatment for MRSA. Hence, the wide use of VA made the development of resistance against this drug a significant worry.[42,48] The previous studies documented the fact that VA resistance genes can transfer horizontally from *E. faecalis* to *S. aureus* and grant it high-level VA resistance. *E. faecalis* has confirmed to be a therapeutic challenge due to it has the capacity for acquisition a broad spectrum of antibacterial resistance agents, which became a serious problem in the treatment of enterococcal infections; the VA resistance genes are carried on plasmids. Hence, *E. faecalis* can give and receive these plasmids during the conjugation.[49-52]

CONCLUSION

The emergence of VRE became a great challenge with serious complications of the control policy for nosocomial infections in our hospitals.

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REFERENCES

1. Gilmore M. The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistance. Washington, DC: American Society for Microbiology; 2002.
2. Lebreton F, Depardieu F, Bourdon N, Fines-Guyon M, Berger P, Camiade S, *et al.* D-ala-d-ser vanN-type transferable vancomycin resistance in *Enterococcus faecium*. Antimicrob Agents Chemother 2011;55:4606-12.
3. Patel R, Piper K, Cockerill FR 3rd, Steckelberg JM, Yousten AA. The biopesticide *Paenibacillus popilliae* has a vancomycin resistance gene cluster homologous to the enterococcal vanA vancomycin resistance gene cluster. Antimicrob Agents Chemother 2000;44:705-9.
4. Woodford N, Johnson AP, Morrison D, Speller DC. Current perspectives on glycopeptide resistance. Clin Microbiol Rev 1995;8:585-615.
5. Murphy JE, Gillespie DE, Bateman CV. Predictability of vancomycin trough concentrations using seven approaches for estimating pharmacokinetic parameters. Am J Health Syst Pharm 2006;63:2365-70.
6. Levine DP. Vancomycin: A history. Clin Infect Dis 2006;42 Suppl 1:S5-12.
7. Michael S, Mark P. The Killers Within: The Deadly Rise of Drug-resistant Bacteria. Boston: Little, Brown and Company; 2003.
8. Kouchak F, Askarian M. Nosocomial infections: The definition criteria. Iran J Med Sci 2012;37:72-3.
9. Fong IW, Karl D. Antimicrobial Resistance and Implications for the 21st Century. New York: Springer; 2007.
10. Willems RJ, Top J, van Santen M, Robinson DA, Coque TM, Baquero F, *et al.* Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. Emerg Infect Dis 2005;11:821-8.
11. Al-Sa'ady AT, Naher HS. Study of the routs of etiologic bacteria causing neonatal infections in Al-Hilla city/Iraq. Biochem Cell Arch 2018;18:577-86.
12. Almamori FF, Alzubaidi FA, Al-Sa'ady AT, Hamid G. Drugs as a cause and treatment for tumor eosinophilic cystitis in surgical cases reported. J Pharm Sci Res 2019;11:84-5.
13. MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Baltimore: Williams and Wilkins; 2000. p. 321-400.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
15. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard M100-S20. Vol. 32. Wayne, PA: National Committee for Clinical Laboratory Standards; 2012.
16. Drake DR, Brogden KA. Continuous-culture chemostat systems and flow cells as methods to investigate microbial interactions. In: Brogden KA, Guthmiller JM, editors. Polymicrobial Diseases. Ch. 2. Washington, DC: ASM Press; 2002.
17. Collee JG, Fraser AG, Marmino BP, Simons A. Mackin and McCartney Practical Medical Microbiology. 14th ed. USA: The Churchill Livingstone, Inc.; 1996.
18. Garges HP, Moody MA, Cotten CM, Smith PB, Tiffany KF, Lenfestey R, *et al.* Neonatal meningitis. Pediatrics 2006;117:1094-100.
19. Trotman H, Bell Y. Neonatal sepsis in very low birthweight infants at the university hospital of the West Indies. West Indian Med J 2006;55:165-9.
20. Cohen-Wolkowiez M, Moran C, Benjamin DK, Cotten CM, Clark RH, Benjamin DK Jr., *et al.* Early and late onset sepsis in late preterm infants. Pediatr Infect Dis J 2009;28:1052-6.
21. Alfaleh KM. Incidence of late onset neonatal sepsis in very low birth weight infants in a tertiary hospital: An ongoing challenge. Sultan Qaboos Univ Med J 2010;10:227-30.
22. Youssef DM, Abd-Elfateh H, Sedeek R, Seleem S. Epidemiology of urinary tract infection in neonatal intensive care unit: A single center study in Egypt. J Acad Med Sci 2012;2:25-9.
23. Strabelli TM, Cais DP, Zeigler R, Siciliano R, Rodrigues C, Carrara D, *et al.* Clustering of *Enterococcus faecalis* infections in a cardiology hospital neonatal intensive care unit. Braz J Infect Dis 2006;10:113-6.
24. Samuelsson A, Jonasson J, Monstein HJ, Berg S, Isaksson B. Clustering of enterococcal infections in a general intensive care unit. J Hosp Infect 2003;54:188-95.
25. Fernandes AT, Filho NR, Mazzano RS, Santana LB, Cerbara EF, Cassaro E Jr. Bactériasaeróbias. In: Fernandes AT, Fernandes MA, Filho NR, editor. Hospital-acquired Infection and it's Interfaces in the Area of Healthcare. Vol. 14. São Paulo: Atheneu; 2000. p. 345-6.
26. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.
27. Tendolkar PM, Baghdayan AS, Shankar N. Pathogenic enterococci: New developments in the 21st century. Cell Mol Life Sci 2003;60:2622-36.
28. Lee MC, Rios AM, Aten MF, Mejias A, Cavuoti D, McCracken GH Jr., *et al.* Management and outcome of children with skin and soft tissue abscesses caused by community-acquired Methicillin-resistant *Staphylococcus aureus*. Pediatr Infect Dis J 2004;23:123-7.
29. Rôças IN, Siqueira JF Jr., Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod 2004;30:315-20.
30. Ryan KJ, Ray CG. Sherris Medical Microbiology. 4th ed. New York: McGraw-Hill; 2004.
31. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, *et al.* NHSN annual update: Antimicrobial-resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2006-2007. Infect Control Hosp Epidemiol 2008;29:996-1011.
32. Elander RP. Industrial production of beta-lactam antibiotics. Appl Microbiol Biotechnol 2003;61:385-92.
33. Alvarez-Ortega C, Wiegand I, Olivares J, Hancock RE, Martínez JL. Genetic determinants involved in the susceptibility

of *Pseudomonas aeruginosa* to beta-lactam antibiotics. Antimicrob Agents Chemother 2010;54:4159-67.
34. Al-Fu'adi AH. Phenotypic and Genotypic (*mec*A gene) of Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates in Dewaniya City. M.Sc Thesis. College of Medicine, Babylon University; 2010.
35. Watanabe A, Takahashi H, Kikuchi T, Kobayashi T, Gomi K, Fujimura S, *et al*. Comparative *in vitro* activity of S-4661, a new parenteral carbapenems, and other antimicrobial agents against respiratory pathogens. Antimicrob Agents Chemother 2000;46:184-7.
36. Nomura S, Nagayama A. *In vitro* antibacterial activity of S-4661, a new parenteral carbapenem, against urological pathogens isolated from patients with complicated urinary tract infections. J Chemother 2002;14:155-60.
37. Mitscher LA. Bacterial topoisomerase inhibitors: Quinolone and pyridone antibacterial agents. Chem Rev 2005;105:559-92.
38. Al-Hassnawi HH. Molecular Characterization of Antibiotic Resistance and Virulence Factors of Methicillin Resistance *Staphylococcus aureus* (MRSA) Isolated from Clinical Cases in Babylon Province. PhD. Thesis. College of Medicine. University of Babylon; 2012.
39. Hussain FM, Boyle-Vavra S, Bethel CD, Daum RS. Current trends in community-acquired Methicillin-resistant *Staphylococcus aureus* at a tertiary care pediatric facility. Pediatr Infect Dis J 2000;19:1163-6.
40. Sattler CA, Mason EO Jr., Kaplan SL. Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible *Staphylococcus aureus* infection in children. Pediatr Infect Dis J 2002;21:910-7.
41. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, *et al.* Guidelines for the laboratory diagnosis and susceptibility testing of Methicillin-resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother 2005;56:1000-18.
42. Panesso D, Ospina S, Robledo J, Vela MC, Peña J, Hernández O, *et al.* First characterization of a cluster of vanA-type glycopeptide-resistant *Enterococcus faecium*, Colombia. Emerg Infect Dis 2002;8:961-5.
43. Floss HG, Yu TW. Rifamycin-mode of action, resistance, and biosynthesis. Chem Rev 2005;105:621-32.
44. Abbas EC, Al-Saady AT, Al-Khafaji ZA, Kadhum MJ. Association of IL-17 with hepatitis A virus childhood infection patients less than 10 years old. J Pharm Sci Res 2018;10:1165-6.
45. Lodise TP, Patel N, Lomaestro BM, Rodvold KA, Drusano GL. Relationship between initial vancomycin concentration-time profile and nephrotoxicity among hospitalized patients. Clin Infect Dis 2009;49:507-14.
46. Pootoolal J, Neu J, Wright GD. Glycopeptide antibiotic resistance. Annu Rev Pharmacol Toxicol 2002;42:381-408.
47. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417-33.
48. Robledo C, Robledo J. Overview of antibiotic resistance in Colombia. In: Salvatierra-Gonzalez R, Benguigui Y, editors. Antimicrobial Resistance in the Americas: Magnitude and Containment of the Problem. Washington, DC: Pan American Health Organization; 2000. p. 134-41.
49. Acar J, Casewell M, Freeman J, Friis C, Goossens H. Avoparcin and virginiamycin as animal growth promoters: A plea for science in decision-making. Clin Microbiol Infect 2000;6:477-82.
50. Paulsen IT, Banerjei L, Myers GS, Nelson KE, Seshadri R, Read TD, *et al.* Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science 2003;299:2071-4.
51. Lauderdale TL, Shiau YR, Wang HY, Lai JF, Huang IW, Chen PC, *et al.* Effect of banning vancomycin analogue avoparcin on vancomycin-resistant enterococci in chicken farms in Taiwan. Environ Microbiol 2007;9:819-23.
52. Anitha B, Surekha V, Mahalakshmi K, Mohan V. Gram-positive microorganisms in periodontitis. J Drug Invent Today 2019;12:1199-203.

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