Ibn Al-Haitham Journal for Pure and Applied science https://doi.org/ 10.30526/2017.IHSCICONF.1777

Antimicrobial susceptibility of Enterococcus spp. Isolated from different clinical sources in Kirkuk provency

Hajir Ali Shareef
Shara Najmalddin Abdullah
Shareefalill23@gmail.com
University of Kirkuk –College of Medicine.

Abstract

One of the most important problems confronts hospitals is the strains emergence of *Enterococcus spp*. with multiple resistance to antibiotics, which propel researchers to modify or produce new antibiotics or combination between two antibiotics so that to be more effective against *Enterococcus*. This study was aimed to susceptibility some of local *Enterococcus spp*. Isolates with of 21 antibiotic using disc diffusion method. The results showed absolute resistant 100% toward (Cephalexin, Gentamycin, Amikacin, Erythromycin and Nalidixic acid), while showed a high sensitivity toward (Vancomycin and Impenem) at percentage of 92.3% for each . Also highly inhibitory activity was observed by using penicillins antibiotics groups against most *Enterococcus* isolates. which contribute to that none of the isolates showed it is ability to produce beta – lactamase enzymes by iodometric tube method. Also susceptibility to some new and synergetic antibiotic like Gentamicin High level(synergy), Streptomycin High level (synergy), Linezolid, Tigecycline, Levofloxacin, Quinupristin /Dalfopristin was conducted by Vitek-2 system. the results showed the absolute sensitivity (100%) of isolates toward (Linezolid and Tigecycline).

All isolates showed multiple –resistant prescription to antibiotics, the number of antibiotics that every isolates resisted range between 6-12 antibiotic.

Key words: *Enterococcus spp.*, Antibiotic susceptibility, Multi-drug resistance ,Linezolid , Tigecycline.

1. Introduction

Enterococcus genus contains many species cause infections to human. *E.faecalis* is one of the most common species associated with nosocomial infections accounting for 90% of the clinical isolates followed by *E. faecium* which in recent years has shown an increase in prevalence. This may be due to that *E. faecalis* has most of the virulence factors of this genus, and *E.faecium* was characterized by the multiple resistance properties of antibiotics [1; 2; 3].
Enterococcus have become increasingly important not only because of their hospital infection injury but because of the increase in the prevalence of resistance to many antibiotics that have becomes causes common health problems globally as well as incidence of treatment failure due to the speed of antibiotic resistance through bacterial conjugations mechanisms [4; 5; 6].
Antibiotics that show varying degrees of efficacy against in-vitro *Enterococcus spp.* includes penicillins (penicillin, ampicillin, bipercillin), glycopeptides (vancomycin and ticoplanin), carbapenems (Imipenem and miropenem), aminoglycosides (gentamycin and streptomycin), tetracycline (tetracycline and oxycycline) and quinolones (Ciprofloxacin, gatifloxacin, gemifloxacin), chloramphenicol, rifampcin, Streptogramin (quinupristin / dulfopristin (Q / D)) , and Oxazolidinme and linezolid antibiotics [7].
Most *Enterococcus* infections are treated with one type of antibiotic, especially in patients with a normal immune system. UTIs is treated with ampicillin, if strain resistant ampicillin, vancomycin is used. If it resistant both of them, linezolid used as an alternative treatment, but in severe cases it is preferable to use acombination of Beta-lactam antibiotics with aminoglycosides such as Nitrufurantion with Fasfomycin, as it shown a strong In-vitro effect against *Enterococcus spp.* [7; 8].
In the last decade, several antimicrobial agents have shown a 70% inhibitory effect against multiple-resistance *Enterococcus* and have been successfully used to treat the infections caused by this microbial, including Streptogramin (Quinupristin-dalfopristin (Q / D)), Daptomycin , Linezolid and Tigecyclin, which are used to treat skin and soft tissue infections and abdominal cavity infections [9; 10; 11].

2. Material and Methods

Samples

A total of 39 clinical isolates of *Entrococcus spp*. Including (E. *faecalis, E. faecium ,E. gallinarum and E. durans)* were isolated from sample of different clinical soures of reviewed patients to Kirkuk hospitals .All isolates identification were done by standard methods including biochemical tests ,serological identification by lancefield methods, identification of Enterococcus species by AP system and Vitek-2 system [12; 13; 14].

Antibiotic susceptibility test

Isolates under study was investigated for their susceptibility against 21 antibiotics include (Penicillin G , Ampicillin , Amoxicillin , Augmentin , Cefotaxime , Ceftriaxone , Cephalexin , Imipenem ,Tetracycline , Rifampcin , Gentamycin , Amikacin , Erythromycin , Vancomycin , Chloramphenicol , Trimethoprim , Co-Trimoxazole , Nitrofurantion , Nalidixic acid , Norfloxacin , Ciprofloxacin) by disc diffusion method depending on [15]. The diameter of the inhibition zone was measured on each disk and compared with the measurements of Clinical and laboratory standards institute (CLIS) guideline [16].

Detection of beta-lactamase enzyme

Iodine tube method was used to detect the production of the Beta-lactamase enzymes [17].

3. Results and Discussion

Resistance of Enterococcus to antibiotics

Enterococcus spp. susceptibility to antibiotics are different in studies conducted in all countries, it was observed that the sensitivity varies according to geographical regions and depending on the misuse of these antibiotics [18].

As for the resistance of *Enterococcus* isolates to Beta-lactam, the results in Table 1 showes that penicillins were the most effective against all isolates. The sensitivity to these antibiotics ranged from 89.7% to amoxicillin, 74.3% to penicillin G and ampicillin and 48.71 % to Augmentin, while the cephalosporin antibiotics had low inhibitory effect against all isolates, the resistance to these antibiotics ranged from 92.3% to 97.4% for ceftriaxone and cefotaxime respectively and 100% for cephalexin. These results were consistent with the study of [19] and [20]. The results of the current study were consistent with [6], who recorded a high sensitivity to penicillin.The sensitivity and resistance ratios of these antibiotics varied in many studies in other countries, particularly for penicillin [21; 22; 23].

Acquisition of mobile genetic components such The low resistance of isolates in this study to penicillins may be due to their lack producing of Beta-Lactamase enzyme were confirmed through the detection of these enzymes using the iodine tube method. On the other hand, [5] pointed a high resistance to cephalosporins due to the production of modified pencillin - binding proteins (PBP2a) with low affinity binding to Beta-lactam antibiotic, or because of its as plasmids and others, as well as the intrinsic resistance to these antibiotics.

For the vancomycin a high inhibitory effect 92.3% of isolates study and only two isolates of *E. faecalis* and one isolate of *E. Gallinarum* have shown resistance for this antibiotic. These resultes were consistent with were recorded in many European countries like Denmark, Norway, newzealand and France [24]. Similar resultes recorded in India and Turkey [4; 5]. Countries with high vancomycin resistance *Enterococcui* were Ireland, Germany, Portugal and United Kingdom [16; 25]. In Iraq governorates such as Mosul and Diyala high rates of resistance to vancomycin were recorded between *Enterococcus* isolates [19; 20].

The interest of researchers and medicines have increased to Vancomycin – resistant Enterococci (VRE) for several reasons, including: the availability of few therapeutic options to treat VRE infections that may pass from enterococci to other bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus*. Both of [26; 27] certain that when these bacteria are present in the hospital environment they are transmitted to patients and spread directly from one patient to another or indirectly through transport by individuals hands or contaminated surfaces of the environment or patient care equipment

As for the remaining antibiotics used in the current study, all isolates showed absolute resistance to both erythromycin and nalidixic acid by 100%, while the sensitivity and resistance ratio for the other antibiotics varied as shown in Table (1), with some consistent to studies that earlier refer to it.

It was noted through the results that there is a pattern of multiple resistance among *Enterococcus* species . The results showed that the isolates of *E.faecalis* were able to resist (6) antibiotics of the total (21) antibiotics, while *E.faecium* and *E.gallinarum* were able to resist (8) antibiotics,while *E.durans* had more ability to resist 12 types of the same total antibiotics were used. This result was consistent with [20]. The multi-resistance is a major threat to the life· s of infected patients and is a source of Large concern for all members of the community because the transmission of infection caused by these isolates is occur through close contact .

One of the most important problems encounter hospitals is the problem of the emergence of strains of Enterococci with multiple resistance to antibiotics. These problems prompted the researchers to modify or produce new antimicrobial or combining two or more antibiotics to get medicine more effective against *Enterococcus spp*. ,such as combination between penicillin and glycopeptides with aminoglycosides (gentamicin, amikacin, streptomycin, etc.) to treat *Enterococcus spp. i*nfections, and new antibiotics such as Linezolid, Tigecycline, Daptomycin and Dalfopristin / Quinopristin . Several studies have confirmed the high efficacy of these antibiotics against *Enterococcus* isolates from different clinical sources [2; 28; 29; 30].

The present study confirms the effectiveness of these antibiotics when perform sensitivity testing with Vitek 2 compact system . All the isolates studied showed their absolute sensitivity to Linezolid, Tigecycline and Dalfopristin / Quinopristin, while showed a sensitivity of 97.5% to the Streptomycin High Level Synergy (HLS) and one isolates of *E. faecalis* was resist it, while the ratio of sensitivity to Gentamicin High Level Synergy (HLG) was 51.28% for 18 isolates of *E. faecalis* whil one isolate from both *E.gallinarum* and *E. durans* resist it Table (3).

These results are consistent with many studies concerning the first three antibiotics mentioned above [5; 6; 16] also came into conformity with a number of studies that *Enterococcus* isolates showed resistant to High level resistance Aminoglycosidase (HLAR) as a study [31] in Iran and the study researchers [19; 27] in India, study researchers [5; 32] in Turkey and study [16] in serbia. The researchers [6; 31] noted that resistance to (HLAR) varies from 1-49% with rate of 22.6-12.3 in 27countries in Europe. HLAR recorded 50% ratio between *E.faecalis* isolates in Italy and 49.5% in Slovakia, 48.6% in Hungary, 48.4% ratio in Poland [24].

In addition to self-resistance to low concentrations of aminoglycosides, *Enterococcus spp.*can acquire resistance against high concentrations of these antibiotics. The mechanism of resistance is due to the presence of genetic elements on the cell chromosome that encode to modified inhibitor enzymes such as aminoglycoside acetyltransferase, AAC (6) –Ii , these enzymes act to alter the amino-group or carboxylic group of aminoglycoside and thus eliminate the synergy between the active antibiotics on the cell wall and aminoglycosides. This enzyme gains *Enterococcus spp*. high resistance to all Important aminoglycosides except the Streptomycin [34].

High resistance to antibiotics by microorganisms arise from widespread and indiscriminate use of antibiotics leading to increase the proportion of resistance in bacteria including Enterococcus *spp.* as genetic response in these bacteria as a result of the pressure generated by this use and this makes it difficult to treat the injuries caused by these bacteria [35]. Also both disease severity, quality of antibiotics, misdiagnosis of causing agents and lack sufficient control over pharmacies to limit the sale of direct therapy to citizens without prescriptions and non-compliance with therapeutic session gives rise to high resistance against these antibiotics, especially in developing countries [36].

Ibn Al-Haitham Journal for Pure and Applied science https://doi.org/ 10.30526/2017.IHSCICONF.1777

Table (1): The number and percentage of *Enterococcus* isolates study with response to antibiotics.

Antibiotics	Sensitive isolates		Resistance isolates	
Penicillins	NO	%	NO	%
Penicillin G	**29**	**74.35**	**10**	**25.64**
Ampicilin	**29**	**74.35**	**10**	**25.64**
Amoxicilin	**35**	**89.74**	**4**	**10.2**
Augmentin	**19**	**48.71**	**20**	**51.28**
Cephalosporins				
Cefotaxime	**1**	**2.56**	**38**	**97.43**
Cetriaxone	**3**	**7.69**	**36**	**92.307**
Cephalexin	**0**	**0**	**39**	**100**
Carbapenems				
Imipenem	**36**	**92.307**	**3**	**7.69**
Tetracyclines				
Tetracycline	**5**	**12.82**	**34**	**87.17**
Rifampcins				
Rifampcin	**3**	**7.69**	**36**	**92.307**
Aminoclycosides				
Gentamicin	**0**	**0**	**39**	**100**
Amikacin	**0**	**0**	**39**	**100**
Macrolides				
Erythromycin	**0**	**0**	**39**	**100**
Glycopeptides				
Vancomycin	**36**	**92.307**	**3**	**7.69**
Chloramphenicol	**16**	**41.02**	**23**	**58.92**
Trimethoprime	**21**	**53.84**	**18**	**46.15**
Co-Trimoxazole	**10**	**25.64**	**29**	**74.35**
Nitrofurantion	**34**	**87.17**	**5**	**12.82**
Quinolones				
Nalidixic	**0**	**0**	**39**	**100**
Fluroquinolones				
Norfloxacin	**9**	**23.076**	**30**	**76.92**
Ciprofloxacin	**11**	**28.205**	**28**	**71.79**

Ibn Al-Haitham Journal for Pure and Applied science https://doi.org/ 10.30526/2017.IHSCICONF.1777

Table (2): Sensitivity test by Vitek-2 system for a number of new antibiotics.

Isolates	Sensitivity type	Gentamycin High Level (Synergy)[	Streptomycin High Level (Synergy)[	Levofloxacin,	(Dalfopristin/ Quinopristin)	Linezolid	Tigecycline
E.faecalis N=31	R	**18)58.1(%**	**1) 3.22(%**	**1) 3.22(%**	**-**	**-**	**-**
	S	**13)41.9(%**	**30)96.72(%**	**30)96.72(%**	**31 100)(%**	**31 100)%**	**31 100)(%**
E.faecium N=5	R	**5)100(%**	**-**	**-**	**-**	**-**	**-**
	S	**-**	**5) 100(%**	**5)100(%**	**5)100(%**	**5)100%**	**5) 100(%**
E.gallinarium N=2	R	**1) 50(%**	**-**	**-**	**-**	**-**	**-**
	S	**1)50(%**	**2)100(%**	**2)100(%**	**2)100(%**	**2)100%**	**2) 100(%**
E.durans N=1		**R**	**S**	**S**	**R**	**S**	**S**

Table (3): Pattern of multi-drug resistance in *Enterococcus spp.*

Isolates÷	Type of antibiotics resist to it	MDR	
		NO	%
E.faecalis	**E,NA,AK,GN,CLCTX**	**6**	**28.59**
E.faecium	**AK,GN,RA,CL,CRO,CTX,E,NA**	**8**	**38.1**
E.gallinarium	**NA,AK,RA,CL,CRO,CTX,E**	**7**	**33.33**
E.durans	**AK,GN,RA,TE,CL,AUG,AMP,CTP,NIT,SXT,E,NA**	**12**	**57.1**

AMP : Ampicillin , , AUG : Augmentin , CXT : Cefotaxime ,CRO : Ceftriaxone , CL : Cephalexin , TE :Tetracycline RA : Rifampcin , GN : Gentamycin , AK : Amikacin , E : Erythromycin , NIT : Nitrofurantion ,NA : Nalidixic acid , CTP : Ciprofloxacin

References

[1] G.Giraffa,. Enterococci from foods. FEMS Microbiology Reviews, 26, 163-171. 2002
[2] E.W. Koneman, S.P.Allen, W.C. Janda, .Color Atlas and Text Book of Diagnostic Microbiology. 6th ed. Lippincott-Willams and Wilkins . 2006
[3] Y. Sharifi, A.Hasani, R.Ghotaslou, M. Aghazadeh, M. Milani, A. Bazmany, Virulence and Antimicrobial Resistance in Enterococci Isolated from urinary tract infections. 3 (1) : 197-201. 2013
[4] S. Sreeja, PRS. Babu, AG. Prathab, .The prevalence and the characterization of the enterococcus species from various clinical samples in the tertiary care hospital. Journal. Clin. Diagn. Res. 6(9):1486-1488. 2012

[5] L .Shah, S .Mulla, Patel KG, S .Rewadiwala .Prevalence of enterococci with higher resistance level in a tertiary care hospital. a matter of concern. Natl. J. Med. Res. 291:25-27. 2012
[6] RS .Shinde, GV .Koppikar, S.Oommen .Characterization and antimicrobial susceptibility pattern of clinical isolates of Enterococci at a tertiary care hospital in Mumbai, India. Ann Trop Med Publ Health, 5(2):85-88. 2012
[7] M.J. Zervos , M. D. Joseph , M.D. Chow , Annechen , M.D.; R.Robert , M.D. Muder ,. Enterococcus Species . infectious disease and Antimicrobial agents. 2010
[8] MB Perri, E.Hershberger, M .Ionescu, C .Lauter, MJ..Zervos .In vitro susceptibility of vancomycin-resistant enterococci (VRE) to fosfomycin. Diagn Microbiol Infect Dis. Apr;42:269-71 . 2002
[9] Enoch, D.A., J.M. Bygott, M.L. Daly, et al. (2007). Daptomycin. The Journal of infection. 55(3): p. 205-13.
[10] E.Tasina, , A.B. Haidich, S. Kokkali, et al.Efficacy and safety of tigecycline for the treatment of infectious diseases: a meta-analysis. The Lancet infectious diseases. 11(11): p. 834-44. 2011
[11] D.Yahav, A. Lador, M. Paul, et al. Efficacy and safety of tigecycline: a systematic review and meta-analysis. The Journal of antimicrobial chemotherapy, 66(9): p. 1963-71. 2011
[12] J. G. Collee, A. G.Fraser, Marmion, B. P. and A .Simmons,. Mackie and McCartney. Practical Medical Microbiology. 14th ed. Churchill Livingstone , UK .1996
[13] B. A. Forbes, D. F. Sahm, and A. S. Weissfeld, Baily and Scotts .Diagnostic Microbiology.11th ed.Mosby,company Baltimore. USA. 2002
[14] R.M. Atlas, Handbook of Microbiological Media .4th ed ., Taylor and Francis Group, LLC. 2010
[15] P .Vandamme, E .Vercauteren, C .Lammens, Pensart N, Leven M, Pot B, et al. Survey of enterococcal susceptibility patterns in Belgium. J Clin Microbiol.34(10):2572–2576. 1996
[16] Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically approved standard. M07-A8. 18th ed. Vol. 29. Wayne, Pa, USA: CLSI. 2009
[17] K.Thomas, and M.Barbara , Applications in General Microbiology. laboratory Manual 6th ed .489. 2003
[18] M.Mira, M.Deana, J.Zora, Vero, G.; Biljana, M., R.Biljana, .Prevalence of different enterococcal species isolated from blood and their Susceptibility to antimicrobial drugs in Vojvodina, Serbia, 2011-2013. African journal of microbiology research. 8(8): 819 - 824. 2014
[19]Z.H.M.AL-Azzawi . Study on virulence factors and antimicrobial response among enterococci isolated from paients .M.Sc. Thesis, University of Diala . college of Education. 2008
[20] N.SH.Hanno. Astudy on Diagnosis and pathogenicity of Enterococcus isolated from Urinary Tract infection and its ability to cause experimental endocarditis . PH.D. Thesis , University of Msul , college of science. 2002
[21] S .Jain, A .Kumar, Kashyap B, Kaur JR .Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia fro tertiary care hospital in Delhi. Int. Journal. App. Basic. Med. Res.1:80- 83. 2011
[22] S .Mulla , K.G. Patel , T.Panwala , Rewadiwala,S. The Prevalence of Enterococci with a higher resistance Level in a tertiary care hospital:Amatter of Concern . National J Med Res .2(1):25-7. 2012

[23] P .Doddamani, K., Srikanth., T.Nandini, R.Rajagopalan, (Prevalence and Antibiogram of Enterococcus Species in a Tertiary Care Hospital).World Journal of Pharmacy and Pharmaceutical Sciences, 2 (5), 2689-2698. 2013
[24] European Centre for Disease Prevention and Control (2012). Antimicrobial resistance surveillance in Europe 2011. Annual Report of the European Antimicrobial Resistance Network (EARS-Net). Stockholm; ECDC:59-63.
[25] C.A.Arias, G.A.Contreras, Murray, B.E. Management of multidrug-resistant Enterococcal infections. Clin. Microbiol. Infect., 16,555-562. 2010
[26] Cetinkaya, Y.; Falk, P. and Mayhall, C. G. (2000) . Vancomycin – resistant Enterococci. Clin. Microbiol. Rev., 13 (4): 686 – 707.
[27] S .Sood, M .Malhotra, Das BK, A .Kapil .Enterococcal infection and antimicrobial resistance. Indian Journal. Med. Res.128:111-121. 2008
[28] DY.Aksoy, S.Unal, .New antimicrobial agents for the treatment of Gram-positive bacterial infections. Clin. Microbial. Infect. 14:411-420 .2008
[29] M .Yemisen, A .Demirel, Mete B, Kaygusuz A, Mert A, Tabak F, Ozturk R Comparative in vitro antimicrobial activity of tigecyclin against clinical isolates of vancomycin-resistant enterooccusz. Indian. J. Med. Microbiol. 27:373-374. 2009
[30] HY .Tsai, CH .Liao, Chen YH, Lu PL, Huang CH, Lu CT, Chuang YC, Tsao SM, Chen YS, Lui YC, Chen WY, Jang TN, Lin HC, Chen CM, Shi ZY, Pan SC, Yang JL, Kung CE, Liu CE, Cheng YJ, Liu JW, Sun W, Wang LS, Ko WC, Yu KW, Chiang PC, Lee MH, Lee CM, Hsu GJ, Hsueh PR .Trends in susceptibility of vancomycin-resistant Enterococcus faecium to tigecyclin, daptomycin and linezolid and molecular epidemiology of the isolates: results from the tigecyclin in vitro surveillance in Taiwan study, 2006 to 2010. Antimicrob. Agents Chemother. 56(6):3402-3405. 2012
[31] M.Talebi, F.Rahimi, M.Katouli, I. Ku¨hn, Mo¨llby, R., Eshraghi, S. and Pourshafie, M.R. Prevalence and antimicrobial resistance of enterococcal species in sewage treatment plants in Iran. Water Air Soil Pollut 185, 111–119. 2007
[32] G.Baldir, D.O. Engin, M.Kucukercan , A.Inan , Akcay,S ., Ozyurek ,S.Aksaray , S. High-Level resistance to aminoglycoside ,Vancomycin,and Linezolid in enterococcus Strains.Journal of Microbiology and Infectious Diseases / JMID.3(3):100-103.2013
[33] J.Parameswarappa, V.P.Basavaraj, C.M.Basavaraj. Isolation, Identification, and antibiogram of enterococci isolated from patients with urinary tract infection. Ann of African Med. 12(3) : 176 – 181 . 2013
[34] T.C.S. Rosvoll . Plasmid , Resistance and Hospital adaptation in Enterococci. an epidemiological approach. Faculty of health sciences. Department of medical biology. 2012
[35] IN .Okeke, A .Lamikanra, R.Edelman .Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerging Infectious Diseases. 5:18–27. 1999
[36] RE .Istúriz and C .Carbon .Antibiotic use in developing countries. Infect. Control Hosp. Epidemiol.,21(6): 394-397. 2000