Antimicrobial Susceptibility Patterns of Enterococcal Isolates And its Relevance With Biofilms Formation And B -Lactamase Production

*Abdul-Razak SH. Hasan (Ph.D.)
**Abbas A. Al-Duliami (Ph.D.)
***Kariem S. Al-Ajeely (Ph.D.)
****Zainab H. Al-Azawi (M.Sc.)
Diyala, Iraq.

Abstract

Background: Enteroccci are part of the normal intestinal flora of human and animal, but with increasing antimicrobial resistance, enterococci are recognized as serious nosocomial as well as community pathogens.

Objectives: To investigate the antimicrobial susceptibility patterns of 44 isolates of enterococci recovered from different pathological specimens from in-and out-patients from Diyala province.

Materials and methods: The present study was conducted in Baquba General Hospital and Al-Batool Hospital for Maternity and children during the period from 1st. September/2005 to 30th. September /2006. A total of 343 specimens were collected from 213 inpatients and 130 outpatients. 200 (58.3%) were females and 143 (41.7%) were males. The mean age of patients was (32.8 ± 17.2) years. Specimens include, urine, stool, vaginal swabs, throat swabs, burn swabs, blood for culture, middle ear swabs, wound swabs, sputum and cerebrospinal fluid. Specimens were streaked on blood agar, and other differential and selective media. 44 isolates of enterococci (30 E. faecalis, 10 E. faecium, 3 E. gallinarium, and 1 E. avium) were recovered and identified according to standard bacteriological and biochemical criteria. The susceptibility patterns toward 13 antimicrobial agents were done by disc diffusion method. Data were statistically analysed.

Results: The results revealed that the highest susceptibility of enterococcal isolates was toward the Nalidixic acid (79.5%), Ciprofloxacin (61.4%), Amoxacillin+clavilanic acid (61.4%), Rifampicillin (36.4%),Trimethoprim (22.7%), Vancomycin (11.4%). However, all isolates were resistant to Cloxacillin, Cefotaxim, Amoxicillin, Tetracycline, and Erythromycin. The susceptibility of non- β -lactamase producing isolates to penicillin were significantly higher than β - lactamase producing isolates ($p<0.001$). Furthermore, the sensitivity of non-biofilms former isolates were significantly higher than that of biofilms former isolates ($p= 0.002$).

Conclusion: The overall susceptibility rates of enterococcal isolates recovered from nosocomial as well as community acquired infections to available antimicrobials are low.

Keywords: Enterococci, E. faecalis, Antimicrobial susceptibility.

*College of Medicine- Diyala University
**College of Education- Dyala University
***Colege of Vet. Medicine-Diyala University
****College of Education- Diyala University
Diyala, Iraq.

Introduction

Enterococci are widely distributed in the environment, they are normal commensals that usually inhabit the alimentary tract of human in addition to being isolated from environmental and animal sources [1,2]. E. faecalis and E. faecium are the most prevalent species cultured from human, accounting for more than 90% of clinical isolates. Other enterococci species to cause human infection include, E. avium, E. gallinarum, E. casseliflavus, E. durans, E. raffinosus, and E. mundtii [3-5].

In the past 15 years, enterococci have emerged as increasingly important cause of acquired nosocomial infections worldwide, including urinary tract infection, bacteremia, surgical wound infection, intraabdominal and pelvic infection, endocarditis, and meningitis [6-9]. An alarming fact is the intrinsic resistance to many antimicrobial agents and the acquisition of resistance to other antibiotics available for treatment has led to therapeutic difficulties worldwide [10-12]. Studies on the antimicrobial susceptibility patterns of enterococci have affirmed the worldwide emergence of multiple-drug resistant enterococci, particularly vancomycin [13-17].

It has been documented that nosocomial enterococci have numerous virulence factors that enhance their ability to colonize hospitalized patients, contribute to antimicrobial resistance, and aggravate the outcome [18-20]. Among the virulence factors are biofilms formation and β-lactamase production. It has been reported that biofilms formation capacity is restricted to enterococci harboring enterococci surface protein which promotes primary attachment and biofilms formation [21,22]. On the other hand, β-lactamase producing enterococci have acquired resistance to penicillins, ephalosporins, carbapenems and monobactams [23,24].

Materials and methods

The present study was conducted in Baquba General Hospital and Al-Batool Hospital for Maternity and children during the period from 1st. September/2005 to 30th. September /2006. A total of 343 specimens were collected from 213 inpatients and 130 outpatients. 200 (58.3%) were females and 143 (41.7%) were males. The mean age of patients was (32.8 ± 17.2) years. Specimens include, urine, stool, vaginal swabs, throat swabs, burn swabs, blood for culture, middle ear swabs, wound swabs, sputum and cerebrospinal fluid. Specimens were streaked on blood agar, and other differential and selective media. 44 isolates of enterococci (30 E. faecalis, 10 E. faecium, 3 E. gallinarium, and 1 E. avium) were recovered and identified according to standard bacteriological and biochemical criteria. The ability of β-lactamase production was detected according to the method described by [25]. Detection of biofilms formation was followed the method of [26].The susceptibility patterns toward 13 antimicrobial agents were done by disc diffusion method. Determination of sensitive or resistant antimicrobial was based on National Committee for Clinical Standards (NCCLS) [27]. Data were statistically analyzed.

Results

The results in table (1) revealed that the highest sensitivity rate of enterococci isolates was toward the Nalidixic acid (79.5%), followed by Ciprofloxacin, Amoxacillin+clavilanic acid (61.4%), Rifampicillin (36.4%),Trimethoprim (22.7%), vancomycin (11.4%). However, all isolates were resistant to Cloxacillin, Cefotaxim, Amoxicillin, Tetracycline, and Erythromycin.

Table 1: Antimicrobial susceptibility of enterococcal isolates.

Antimicrobials	Enterococcal isolates				Total (n=44)
	E. faecalis (n=30)	E. faecium (n=10)	E. gallinarium (n=3)	E. avium (n=1)	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Vancomycin	0(0)	2(20)	3(100)	0(0)	5(11.4)
Ciprofloxacin	18(60)	7(70)	1(33.3)	1(100)	27(61.4)
Rifampicin	11(36.7)	5(50)	0(0)	0(0)	16(36.4)
Nalidixic acid	25(83.3)	7(70)	2(66.7)	1(100)	35(79.4)
Penicillin	7(23.3)	4(40)	0(0)	1(100)	12(27.3)
Amoxacillin + clavulanic acid	16(53.3)	8(80)	3(100)	0(0)	27(61.4)
Trimethoprim	6(20)	3(30)	1(33.3)	0(0)	10(22.7)

Table (2) showed the antimicrobial susceptibility patterns of E. faecalis in comparison with other enterococcal isolates. All isolates of E. faecalis were resistant to vancomycin, while 5(35.7%) of other enterococcal isolates were sensitive to it. The difference between the two groups was statistically significant (p=0.002).

Table 2: Antimicrobial susceptibility according to enterococcal species.

Antimicrobials	Enterococcal isolates		P (Fisher's exact)
	Other species (n=14)	E. faecalis (n=30)	
	No. (%)	No. (%)	
Vancomycin	5(35.7)	0(0)	0.002 [S]
Ciprofloxacin	9(64.3)	18(60)	1 [NS]
Rifampicin	5 (35.7)	11(36.7)	1 [NS]
Nalidixic acid	10 (71.4)	25 (83.3)	0.43 [NS]
Penicillin	5 (35.7)	7 (23.3)	0.43 [NS]
Amoxacillin + clavulanic acid	11 (78.6)	16 (53.3)	0.18 [NS]
Trimethoprim	4(28.6)	6 (20)	0.7 [NS]

Regarding the effect of β-lactamase production on susceptibility to antimicrobial agents, the results revealed that the susceptibility of non- β -lactamase producing isolates to penicillin were significantly higher than β - lactamase producing isolates (p<0.001). Additionally, the resistance of β - lactamase producing isolates to Vancomycin, Ciprofloxacin, Rifampicin Nalidixic acid and Trimethoprim were insignificantly higher than that of non β - lactamase producing isolates. Moreover, the sensitivity of β - lactamase producing isolates to Amoxacillin + clavulanic acid was insignificantly higher than non β -lactamase producing isolates, table (3).

Table 3: Antimicrobial susceptibility according to β-lactamase production.

Antimicrobials	β -lactamase production		P (Fisher's exact)
	Non-producer (n=12)	producer (n=32)	
	No. (%)	No. (%)	
Vancomycin	1(23.1)	4(12.5)	1 [NS]
Ciprofloxacin	8(66.7)	19(59.4)	0.74 [NS]
Rifampicin	5(41.7)	11(34.4)	0.73 [NS]
Nalidixic acid	10(83.3)	25(78.1)	1 [NS]
Penicillin	11(91.7)	1(3.1)	< 0.001 [S]
Amoxacillin + clavulanic acid	7(58.3)	20(62.5)	1 [NS]
Trimethoprim	3(25)	7(21.9)	1 [NS]

The results also showed that the sensitivity of non-biofilms former isolates were significantly higher than that of biofilms former isolates (p= 0.002). While the sensitivity to Amoxacillin + clavulanic acid was insignificantly higher in non-biofilms former compared to biofilms former isolates. On the other hands, biofilms former isolates were insignificantly more resistant to Rifampicin and Nalidixic acid compared to non-biofilms former isolates.

Table 4: Antimicrobial susceptibility according to biofilms formation.

Antimicrobials	Biofilms formation		P (Fisher's exact)
	Non-formers (n=10)	Formers (n=34)	
	No. (%)	No. (%)	
Vancomycin	0(0)	5 (14.7)	0.57 [NS]
Ciprofloxacin	6(60)	21(61.8)	1 [NS]
Rifampicin	4(40)	12 (35.3)	1 [NS]
Nalidixic acid	9 (90)	26 (76.5)	0.66 [NS]
Penicillin	7 (70)	5 (14.7)	0.002 [S]
Amoxacillin + clavulanic acid	7(70)	20 (58.8)	0.72 [NS]
Trimethoprim	2(20)	8 (23.5)	1 [NS]

Discussion

The results showed that 79.5% of all enterococcal isolates (100% E. faecalis , 80% E. faecium and 100% E. gallinarium) were resistant to vancomycin. These results are consistent with previous studies [15-17]. The vancomycin resistant enterococci (VRE) have caused hospital outbreaks worldwide, and the vancomycin resistant gene (vanA) has crossed genus boundaries to methicillin resistant Staphylococcus aureus (MRSA). Spread of VER therefore represents an immediate thread for patients care and creates a reservoir for mobile resistance genes for other, more virulent pathogens [28, 29]. The first VRE isolates that harbored the van A transposon were identified in 1987 in Europe, and within 10 years VRE represented > 25% of enterococci associated with nosocomial bloodstream infections in USA [30]. Recently, vancomycin resistant rate among E. faecalis and E. faecium were 5.4% and 75.4% respectively in USA [31]. The acquisition of vancomycin resistance by enterococci has seriously affected the treatment and infection control of these organisms. VRE, particularly E. faecium isolates, are frequently resistant to all antibiotics that are effective in the treatment of vancomycin-susceptible enterococci, which leaves clinicians treating VRE infections with limited therapeutic options [10,32].

The β-lactamase producing enterococci have significantly higher rate for penicillin resistance compared to β-lactamase non-

producing isolates. Additionally, 87.5% of β-lactamase producing enterococci were resistant to vancomycin. These results are not unusual and are in concordant with previous reports[23,24]. Moreover, high level gentamicin resistance was documented among β - lactamase producing E. faecalis that are strongly associated with patients of severe underlying diseases [32].

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