Virulence factors of enterococci species isolated from nosocomial and community acquired infections

Abbas A. Al-Duliami , Nadhum G. Nauman , Afak R. Salman , Abdul-Razak SH. Hasan

College of Education- Diyala University , College of Medicine- Diyala University, College of Education- Diyala University , College veterinary- Diyala University.

Receiving Date: 17-02-2011 - **Accept Date:** 14-06-2011

Abstract

Several virulence and pathogenicity factors have been described from enterococci that enhances their ability to colonize patient's tissues, increase resistance to antibiotics, and aggravate the infection outcome**.** The present study aimed to investigate virulence and pathogenicity factors among enterococci species isolated from nosocomial and community acquired infection in Diyala. The 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. 44 isolates of enterococcal species were recovered from different clinical specimens and identified according to standard bacteriological and biochemical criteria. The presence of certain virulence and pathogenicity factors, namely; gelatinase and hemolysin production, biofilms formation, agglutination of erythrocytes, presence of capsule, and adherence to epithelial cells were detected. Data were statistically analyzed.

The results showed that all isolates of *E. gallinarium* and *E. avium* were biofilm former compared to 76.7% and 70% of *E. faecalis* and *E. faecium* respectively. Furthermore, all isolates of *E. gallinarium* and 76.7% of *E. faecalis* were β-lactamase producer. Additionally, all isolates of *E. avium* and 76.7% of *E. faecalis* were agglutinated RBCs. The presence of capsule was highest among *E. faecalis* isolates (26.7%). The results also revealed

that all *E. galinarium* and *E. avium* isolates were non-hemolytic. Furthermore, among 12 isolates which express β- hemolysis, 10 (33.3%) and 2 (20%) were *E. faecalis* and *E. faecium* respectively. α-hemolysis were found among 10 (26.7%) isolates of *E. faecalis* and 2 (20%) isolates of *E. faecium*. It can be concluded that Local isolates of enterococci species recovered from different clinical specimens are multi-virulence bacteria.

Keywords: Enterococci, Multiple-drug resistance, Virulence factors.

Introduction

Enterococci are part of the normal intestinal flora of human and animals, but are also important pathogen responsible for serious nosocomial and community acquired infections [1,2]. Although the genus enterococcus include more than 17 species, *Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent species recovered from humans, accounting for more than 90% of clinical isolates [3,4]. Isolation of enterococci resistant to multiple antibiotics has become increasingly common in hospital setting worldwide [5-9].Several virulence and pathogenicity factors have been described from enterococci that enhances their ability to colonize patient's tissues, increase resistance to antibiotics, and aggravate the infection outcomes [10-12].

Molecular studies have identified several genes from enterococci encodes virulence factors. Gene for production of gelatinase was detected in vancomycin resistant enterococci (VRE) and vancomycin sensitive enterococci (VSE) in 3.7% and 55.5% respectively [13]. Enterococcal surface protein (*esp*) gene was detected in 71% of *E. faecium* isolates, and in 73% of *E. faecalis* isolates [14]. In another study, *esp* was found in 46.3% and 62.9% of VSE and VRE respectively [13]. Klibi *et al*. (2007)[15] found that 41.2% of *E. faecalis* had cytolysin (*cyl*) gene, and 64% of them showed β-hemolysis. Furthermore, 26.5% and 58% of *E. faecalis* and *E. faecium* harbored *esp* gene, and high proportion of *E. faecalis*, in contrast to *E. faecium* showed high ability for biofilms formation and adherence to cells. However, it has been found that neither the presence of *esp* gene nor the ability to produce gelatinase correlates with the

biofilms formation [16]. β-lactamase producing *E. faecalis* was found in 8.2% of isolates recovered from patients, and significantly correlates with high level gentamicin resistance [17].

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 for β-lactamase production was detected according to the method described by [18]. Detection of biofilms formation was followed the method of [19]. Gelatinase production was determined by subculturing of isolates on gelatin agar slants [20]. Data were statistically analyzed.

Results

Table (1) lists the types and frequencies of virulence factors expressed by enterococcal isolates. Among these 77.3% of the isolates expressed the ability for biofilm formation and 72.7% of them was β- lactamase producer. Moreover, 75% of the isolates had the ability to adhere to epithelial cells. On the other hand, the majority of isolates (79.6%) were uncapsulated.

Table(1): Virulence factors produced by enterococci isolates (n=44).

Property	Negative		Positive	
	No.	%	No.	%
Gelatinase production	13	29.5	31	70.5
B-lactamase production	12	27.3	32	72.7
Biofilms formation	10	22.7	34	77.3
Adherence with epithelial cells	11	25	33	75
Agglutination of RBCs	16	36.4	28	63.6
Presence of capsule	35	79.6	9	20.4

Table (2) revealed the distribution of virulence factoirs according to the enterococcal species. All isolates of *E. gallinarium* and *E. avium* were found to be biofilm former compared to 76.7% and 70% of *E. faecalis* and *E. faecium* respectively. Furthermore, all isolates of *E. gallinarium* and 76.7% of *E. faecalis* were β-lactamase producer. Additionally, all isolates of *E. avium* and 76.7% of *E. faecalis* were agglutinated RBCs. The presence of capsule was highest among *E. faecalis* isolates (26.7%) compare to other enterococcal isolates.

Table (2): Distribution of virulence factors according to enterococcal species.

Property	**Species of isolated enterococci**							
	E. faecalis (n=30)		*E. faecium* (n=10)		*E. gallinarium* (n=3)		*E. avium* (n=1)	
	No.	%	No.	%	No.	%	No.	%
Gelatinase	25	83.3	6	60	0	0	0	0
B-lactamase	23	76.7	6	60	3	100	0	0
Biofilms	23	76.7	7	70	3	100	1	100
Adherence	24	80	6	60	2	66.7	1	100
Agglutination	23	76.7	4	40	0	0	1	100
Capsule	8	26.7	1	10	0	0	0	0

Regarding the hemolysin production, the results revealed that all *E. galinarium* and *E. avium* isolates were non-hemolytic. Furthermore, among 12 isolates which express β-hemolysis, 10 (33.3%) and 2 (20%) were *E. faecalis* and *E. faecium* respectively. α-hemolysis were found among 10 (26.7%) isolates of *E. faecalis* and 2 (20%) isolates of *E. faecium*, table (3).

Table (3): Distribution of hemolysin production according to enterococci species.

Type of hemolysis	**Species of enterococci**								Total (%)
	E. faecalis		*E. faecium*		*E.gallinarium*		*E. avium*		
	No.	%	No.	%	No.	%	No.	%	
Alpha	8	26.7	2	20	0	0	0	0	10 (22.7)
Beta	10	33.3	2	20	0	0	0	0	12 (27.3)
Gamma	12	40	6	60	3	100	1	100	22(50)
Total	30	100	10	100	3	100	1	100	44(100)

Discussion

Enterococci are an important global cause of nosocomial infections, being increasingly associated with urinary tract infections, endocarditis, intra-abdominal and pelvic infections, catheter-related infections, surgical wound infections, and central nervous system infections [1,2]. Several virulence and pathogenicity factors have been described from enterococci that enhance their ability to colonize patient's tissues, increase resistance to antibiotics, and aggravate the infection outcomes [10-12]. Among these virulence factors, the present study found that overall 70.5% of the enterococcal isolates and 83.3% of *E. faecalis* were gelatinase producer. These results are in agreement with other studies which detect the *gel* gene in a range of 40%-70% among enterococcal strains [18-21]. However, one study found that 100% of *E. faecalis* isolates harbored the *gel* gene [22]. Interestingly, it has been reported that the gene detectioin rate was higher among isolates recovered from hospital infections compared to those isolates recovered from healthy volunteers [23].

Except for *E. avium*, the majority of other enterococcal species included in this study were found to be β-lactamase producer at higher levels. Upon reviewing the literature a controversial results have obtained by previous studies; part of these studies which include

different entrococcal species found no β-lactamase activity [24,25]. Other studies found no β-lactamase actvity, although the isolates exhibited high-level aminoglycoside resistance [26,27], or ampicillin resistance [28]. On the other hand, β-lactamase positive, Vancomycin resistant E. faecalis was reported [29]. In India, 34% of clinical isolates of *E. faecalis* were β-lactamase producer [30]. The high rate of β-lactamase production by enterococcal isolates included in the present study probably can be explained by the fact that all of these isolates were recovered from clinical specimens, suggesting that β-lactamase positive enterococci were prevalent in nosocomial infections, that probably arise due to misuse of antibiotics and poor compliance of patients.

Another fascinating result obtained in the current study was that the majority of enterococcal isolates, regardless the species, were biofilm former. This result is consistent with previous studies affirmed the role of biofilm formation in enterococci pathogenesis [18, 30,31]. Of importance, it has been found that *E. faecalis* isolates recovered from UTI with both *asa1* (encoding for aggregation substance) and *esp (*encoding for enterococcal surface protein) genes formed biofilms at significantly higher rates than those with neither gene [20]. Controversially, it has been found that both *E. faecalis* and *E. faecium* did not show a correlation between the presence of either *esp* or the production of gelatinase and biofilm formation [32].

The present results also showed that 100% and 80% of *E. avium* and *E. faecalis* had the ability to adhere to urinary tract epithelial cells *in vitro*. It is worthy to mention that most of the previous studies detect the *asa1* gene coding for aggregation substance and *esp* gene from different enterococcal isolates at different rates [18.19,21,22,33,34]. The higher adhesion rate obtained in the present study may reflect the strong affinity of enterococcal isolates toward the UT epithelial cells, and indirectly explain the higher prevalence of enterococci as a causative agent of nosocomially acquired UTIs.

In the present study, complete hemolysis of RBCs (β-hemolysis) was appeared to be a feature of *E. faecalis* and *E. faecium* isolates but not other species. These results are in agreement with previous studies which yielded a comparable rates of hemolysin activator gene (*cylA*) detection [18,21,31]. Additionally, it has been found that hemolysin was more common in clinical isolates and in hospital fecal isolates than among community fecal

isolates, indicating that intestinal enterococci may differ in this respect from clinical strains [19,23].

References

1. Brooks, G.F.; Carroll, K.C.; Butel, J.S. and Morse, S.A. The streptococci. In: Medical Microbiology. 24th. Ed. 2007. McGraw Hill.233-49.
2. Fisher, K. and Phillips, C. The ecology, epidemiology and virulence of enterococci. Microbiology 2009; 155(6): 1749-57.
3. Hunt, C.P. The emergence of enterococci as a cause of nosocomial infection. Brit. J. Biomed. Sci. 1998; 55(2): 149-56.
4. Akkoyun, S.; Kuloglu. F. and Tokuc, B. Etiologic agents and risk factors in nosocomial urinary tract infections. Microbiol. Bul. 2008;42(2): 245-54.
5. Udo, E.E.; Al-Sweih, N.; John, P. and Chugh, T.D. Antibiotic resistance of enterococci isolated at a teaching hospital in Kuwait. Diag. Microbiol. Infect. Dis. 2002; 43(3): 233-8.
6. Sood, S.; Malhotra, M.; Das, B.K. and Kapil, A. Enterococcal infections and antimicrobial resistance. Indian J. Med. Res. 2008; 128(2): 111-21.
7. Werner. G.; Coque, T.M.; Hammerum, A.M.; Hope, R.; Hryniewicz, W.; et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro. Surveill. 2008; 13(47): 19046.
8. Zhanel, G.G.; DeCorby, M.; Laing, N.; Weshnoweski, B.; Vashisht, R.; et al. Antimicrobial-resistant pathogens in intensive care units in Canada: results of the Canadian National Intensive Care Unit (CAN-ICU) study, 2005-2006. Antimicrob. Agents Chemother. 2008; 52(4): 1430-7.
9. Piroth, L.; Pechinot, A.; Minello, A.; Jaulhac, B.; Patry, I.; et al. Bacterial epidemiology and antimicrobial resistance in ascitic fluid: a 2 years retrospective study. Scand. J. Infect. Dis. 2009; 41(11-12): 847-51.
10. Dupre, I.; Zanetti, S.; Schito, A.M.; Fadda, G. and Sechi, L. Incidence of virulence determinants in clinical Enterococcus faecium and Enterococcus faecalis isolates collected in Sardinia (Italy). J. Med. Micrbiol. 2003; 52(6): 491-8.

11. Marra, A.; Dib-Hajj, F.; Lamb, L.; Kaczmarek, F.; Shang, W.; et al. Enterococcal virulence determinants may be involved in resistane to clinical therapy. Diag. Microbiol. Infect. Dis. 2007; 58(1): 59-65.
12. Dupont, H.; Vael, C.; Muller, C.; Chosidow, D.; Mantz, J.; et al. Prospective evaluation of virulence factors of enterococci isolated from patients with peritonitis: impact on outcome. Diag. Microbiol. Infect. Dis. 2008; 60(3):247-53.
13. Sauer, P.; Sila, J. and Vagnerova, I. Virulence factor in vancomycin-susceptible and vancomycin-resistant enterococci in the University Hospital Olomoucl. Clin. Microbiol. Infect. Lek. 2009; 15(2): 4407.
14. Hallgren, A.; Claesson, C.; Saeedi, B.; Monstein, H.; Hanberger, H. and Nilsson, L. Molecular detection of aggregation substance, enterococcal surface protein, and cytolysin genes and in vitro adhesion to urinary catheters of *Enterococcus faecalis* and *Enterococcus faecium* of clinical origin. Int. J. Med. Microbiol. 2009; 299(5): 323-32.
15. Klibi, N.; Ben Slama, K.; Saenz, Y.; Masmoudi, A.; Zanetti, S.; Sechi, L.; Boudabous, A. and Torres, C. Detection of virulence factors in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus Faecium* isolates from a Tunisian hospital. Can. J. microbial. 2007; 53(3): 372-9.
16. DiRosa, R.; Creti, R.; Vandetti, M.; D'Amelio, R.; Arciola, C.; *et al*. Relationship between biofilms formation, the enterococcal surface protein (Esp) and gelatinase in clinical isolates of *Enterococcus faecalis* and *Enterococcus faecium*. FEMS Microbiol. Lett. 2006; 256(1): 145-50.
17. Wells, V.D.; Wong, E.; Murray, B.; Coudron, P.; William, D.; et al. Infections due to beta-lactamase producing, high level gentamicin resistant *Enterococcus faecalis*. Ann. Intern. Med. 1992; 116(4): 285-92.
18. Archimbaud, C.; Shankar, N.; Forestier, C.; Baghdayan, A.; Gilmore, M.S.; Charbonné, F. and Joly, B. In vitro adhesive properties and virulence factors of *Enterococcus faecalis* strains. Res. Microbiol. 2002;153(2):75-80.
19. Lempiainen, H.; Kinnunen, K.; Mertanen, A. and von Wright, A. Occurrence of virulence factors among human intestinal enterococcal isolates. Lett. Appl. Microbiol. 2005;41(4):341-4.

20. Seno, Y.; Kariyama, R.; Mitsuhata, R.; Monden, K.. and Kumon, H. Clinical implications of biofilm formation by *Enterococcus faecalis* in the urinary tract. Acta Med. Okayama. 2005;59(3):79-87.

21. Dupont, H.; Vael, C.; Muller-Serieys, C.; Chosidow, D.; Mantz, J.; Marmuse, J.P.; Andremon, A.; Goossens, H. and Desmonts, J.M. Prospective evaluation of virulence factors of enterococci isolated from patients with peritonitis: impact on outcome. Diagn. Microbiol. Hinfect. Dis. 2008;60(3):247-53.

22. Klibi, N.; Ben Slama, K.; Sáenz, Y.; Masmoudi, A.; Zanetti, S.; Sechi, L.A.; Boudabous, A. and Torres, C. Detection of virulence factors in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolates from a Tunisian hospital. Can. J. Microbiol. 2007;53(3):372-9.

23. Coque, T.M.; Patterson, J.E.; Steckelberg, J.M. and Murray, B.E. Incidence of hemolysin, gelatinase, and aggregation substance among enterococci isolated from patients with endocarditis and other infections and from feces of hospitalized and community-based persons. J. Infect. Dis. 1995;171(5):1223-9.

24. Kaufhold A, and Klein R. Species identification and antibiotic susceptibility of enterococci isolated from clinical specimens of hospitalized patients. Zentralbl. BaKteriol. 1995;282(4):507-18.

25. Toledo, C.; Perez, M.E.; Rocchi, M.; Gribaudo, G.; Mangiaterra, S. and Monterisi, A. Isolation of enterococci species causative of infections and sensitivity to antimicrobial drugs. Rev. Argen. Microbiol. 2004 ;36(1):31-5.

26. Lam,S.; Singer, C.; Tucci,V.; Morthland, V.H.; Pfaller, M.A.and Isenberg, H.D. The challenge of vancomycin-resistant enterococci: a clinical and epidemiologic study. Am. J. Infect. Control. 1995;23(3):170-80.

27. Kaçmaz, B. and Aksoy, A. Antimicrobial resistance of enterococci in Turkey. Int. J.Antimicrob. Agents 2005;25(6):535-8.

28. Udo, E.E.; Al-Sweih, N.; Phillips, O.A.and Chugh, T.D. Species prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals. J. Med. Microbiol.2003;52(Pt 2):163-8.

29. McAlister, T.; George, N.; Faoagali, J. and Bell, J. Isolation of beta-lactamase positive vancomycin resistant Enterococcus faecalis; first case in Australia. Commun. Dis. Intell.1999;23(9):237-9.
30. Parvathi,S. and Raja B. Comparative evaluation of beta lactamase production in enterococci by acidometric method and clover leaf technique. Indian J. Med. Microbiol. 2000; 18(3): 122-4.
31. Dworniczek, E.; Wojciech, Ł.; Sobieszczańska, B. and Seniuk, A. Virulence of Enterococcus isolates collected in Lower Silesia (Poland). Scan. J. Infect. Dis. 2005;37(9):630-6.
32. Di Rosa, R.; Creti, R.; Venditti, M.; D'Amelio, R.; Arciola, C.R.; Montanaro, L.and Baldassarri, L. Relationship between biofilm formation, the enterococcal surface protein (Esp) and gelatinase in clinical isolates of *Enterococcus faec*alis and *Enterococcus faecium*. FEMS Microbiol Lett. 2006;256(1):145-50.
33. Bittencourt de Marques, E. and Suzart, S. Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. J. Med. Micrbiol. 2004;53(Pt 11):1069-73.
34. Filipová, M. and Bujdáková, H. Factors of virulence and mechanisms of resistance to aminoglycosides in clinical isolates of Enterococcus faecalis and *Enterococcus faecium* with high-level gentamicin resistance. Epidemiol. Mikrobiol. Immunol.2005;54(2):65-7