VANCOMYCIN RESISTANT ENTEROCOCCI ISOLATED FROM PATIENTS ATTENDING AL-KHUMS TEACHING HOSPITAL, AL-KHUMS, LIBYA

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ABSTRACT

Vancomycin resistant enterococci have become a major concern worldwide. The aim of this study is to isolate and identify enterococcal species from different clinical specimens as well as to determine the susceptibility pattern of these isolates to vancomycin. The present study was carried out in two hundred (72 males and 128 females) patients who attended different clinical wards at Al-Khums teaching Hospital, Al-Khums, Libya, during May 2011 to November 2012. All isolated enterococcal species were identified using gram staining and biochemical tests using API-20E system. Antimicrobial susceptibility testing was also performed. The overall enterococcal infection rate was 31.5% of the total specimen examined**.** It was found that out of 37 wound specimens only 5 (13.5%) showed enterococcal infection. The results indicate that the isolation rates of enterococcal isolates are usually more or less equally distributed among both male and female patients concerning throat swabs, sputum and wound specimens. However, the isolation rates of enterococcal isolates from urine specimens were higher in case of female patients (20.5 %) compared with that (6.5%) of male patients. Three different enterococcal species(*Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus avium*) were identified. Antibiotic susceptibility results indicated that 6 isolates *of E. faecalis*, one *isolate of E. ovium* were resistant to vancomycin and no isolates with vancomycin resistant in *E. faecium*.

KEY WORDS: Al-khums, Enterococci, Libya, Resistance, Vancomycin.

INTRODUCTION

Enterococci are enteric gram-positive cocci that constitute part of the normal bacterial flora of the gastrointestinal tract, biliary tracts, vagina and male urethra in human. They are also found in soil, food, water, and as normal flora of animals and birds[(1)]. Enterococci employ many strategies to avoid the inhibitory effects of antimicrobial agents and have evolved highly efficient means for the dissemination of resistance traits, thus evolving potential multidrug resistant pathogens[(2)].

Vancomycin-resistant enterococci have emerged worldwide as important nosocomial pathogens. The prevalence and incidence of vancomycin-resistant enterococci colonization vary widely among hospitals and studies have suggested that such vancomycin-resistant enterococci rates are higher among critically ill patients, particularly those admitted to intensive care units, limiting the therapeutic options available[(3)].

Vancomycin resistance in enterococci is associated with diverse phenotypes and their resistance to several antimicrobial agents, whether intrinsic (low-level resistance to penicillin, cephalosporins and aminoglycosides) or acquired (resistance to glycopeptides and high concentrations of aminoglycosides), is of great concern[(4)].

Vancomycin resistant enterococci have become a major concern worldwide. In the United States, resistance to glycopeptides among enterococci was first noted in the metropolitan hospitals along the eastern seaboard. More recently, this initial pattern has become more geographically diffused. In Europe, glycopeptide resistance has also been detected[(5)].

The aim of this study is to isolate and identify enterococcal species from different clinical specimens as well as to determine the susceptibility pattern of these isolates to vancomycin.

MATERIALS AND METHODS

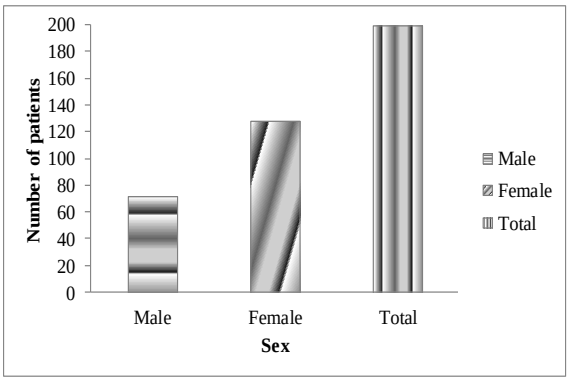
Patients: The present study was carried out on two hundred patients. The study included seventy-two males and one hundred twenty-eight females, from patients attended Al-Khums Teaching Hospital at Al-Khums, Libya, during the period from May 2011 to November 2012. Their age ranged from less than ten years to more than sixty years. Clinical specimens, included in the current study were, swab from infected wounds, throat and sputum from patients suffered from respiratory tract infection as well as urine sample from patients suffering from urinary tract infection. Thirty-seven swabs were collected from post operative wound patients in surgery department, sixteen swabs were obtained from internal medicine department, one hundred forty-one urine specimens were collected from patients attending urology unit and six sputum swab were collected from patients in intensive care unit.

Isolation, identification and susceptibility testing: The samples obtained were transported to the microbiology laboratory for selective culturing of vancomycin-resistant enterococci. The swabs were inoculated on BBLTM Enterococcosel TM Agar plates (Becton and Dickinson Company, France) and incubated aerobically at 35ºC for 48 h. All isolated bac-

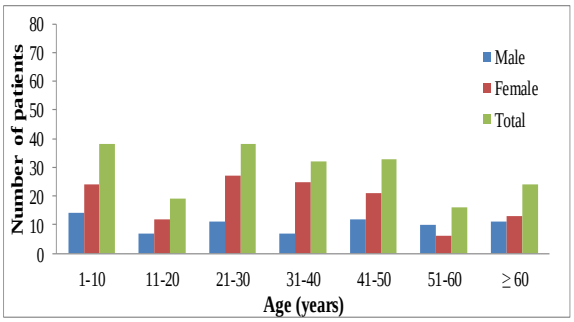
terial species were identified using gram staining and biochemical tests using API-20E (Analytical profile index) system (BioMérieux, S.A. Marcyl'Etoile, France) according to manufacturer's instructions. Antimicrobial susceptibility testing was performed on Müeller-Hinton Agar (BD Company) according to recommendations of the Clinical and Laboratory Standard Institute[6].

RESULTS

Distribution of patients included in the study according to their age and sex were presented in (figure 1 and 2), which showed that patients with age less than 10 years old, 7.0% were male and 12.0% were females, while in age range 11-20 years old 3.5% were males and 6.0% were females. In age range 21-30 years old, 5.5% were males and 13.5% were females, but age range 31-40 years old, 3.5% were males and 12.5 % were females. However, for age range 41-50 years old 6.0 % were males and 10.5 % were females and for age range 51-60 years old, 5.0 % were males and 3.0 % were females. Finally, for age range more than 60 years old, the percentages were 5.5% and 6.5% for male and females respectively.

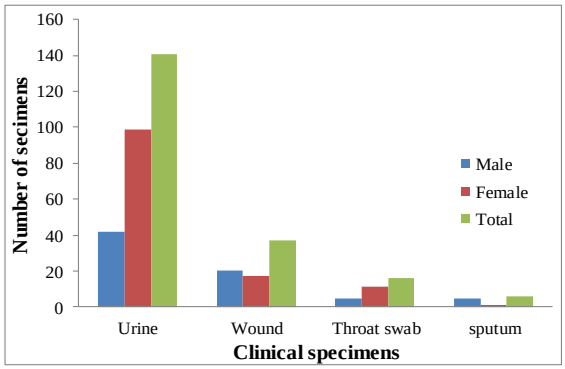


(Figure 1) Distribution of patients according to sex.



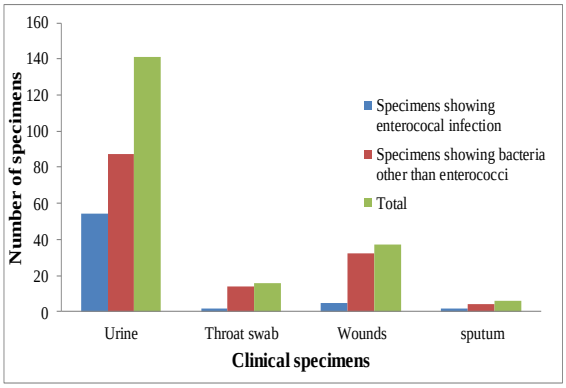
(Figure 2) Distribution of patients according to age.

In the present study, different clinical specimens were subjected to examination. Among these specimens, 141 urine, 37 wound, 16 throat swabs and 6 sputum (figure 3).



(Figure 3) Types and numbers of clinical specimens involved in the study.

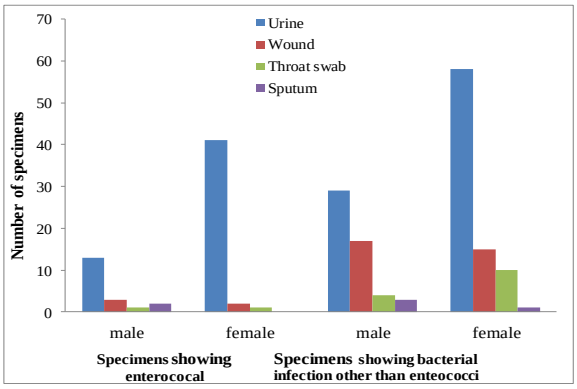
(Figure 4) illustrates prevalence of enterococci in different clinical specimens, in relation to other non-enterococcal pathogens. The overall enterococcal infection rate was 31.5% of the total specimen examined (63 out of 200). Out of 141 urine specimens showed the highest rate; 54 (38.3%) showed enterococcal infection and the other 87(61.7%) showed bacteria other than enterococci, followed by sputum specimens, where out of 6 sputum specimens only 2 (33.3%) showed enterococcal infection. Meanwhile, out of 16 throat swabs examined only, two (12.5%) showed enterococcal infection and the other 14 (87.5%) showed bacteria other than enterococci. Concerning prevalence of enterococcal infection among wound



(Figure 4) Prevalence of enterococci in different clinical specimens.

specimens; it was found that out of 37 wound specimens only 5 (13.5%) showed enterococcal infection.

Prevalence of enterococci in different clinical specimens, in relation to sex of investigated patients was presented in (figure 5). The results indicate that the isolation rates of enterococcal isolates are usually more or less equally distributed among both male and female patients concerning throat swabs, sputum and wound specimens. However, the isolation rates of enterococcal isolates from urine specimens were higher in case of female patients (20.5 %), compared with that (6.5%) for male patients.



(Figure 5) Prevalence of enterococci in different clinical specimens in relation to sex of the patient.

The results of the present study indicated that some enterococcal isolates differ in their hemolytic activities on blood agar as well in their susceptibilities to vancomycin. Concerning the hemolytic activities of enterococcal isolates 36 (57.0%) was α-hemolytic. However, the numbers of γ-(nonhemolytic) enterococcal isolates were 27 (43%) (table 1).

(Table 1) Hemolytic activity of isolated enterococcal isolates.

Hemolytic activity	No	%
α- hemolytic	36	57.0
γ- hemolytic	27	43.0

N.B. % were correlated to total number of enterococcal isolates (63).

Using API 20E strep., three different enterococcal species were identified; thirty-four (54%) enterococcal isolates were identified as *Enterococcus faecalis*, sixteen (25.4%) enterococcal isolates were identified as *Enterococcus faecium* and *thirteen* (20.6%) were identified as *Enterococcus avium*. From these results, it was clear that *Enterococcus faecalis* is more prevalent species among the three different isolated species from the clinical specimens investigated.

Distribution of enterococcal isolates according to the type of clinical specimens was presented in (table 2), urine showed the highest rate (30) (47.6%) of isolation of *Enterocoocus fecalis* isolates followed by throat infection (4) (6.4%). However, *Enterococcus faecium* isolates recovered from (13) (20.6%) of urine specimens, and only two were recovered from wound infection (3.2%). *Enterococcus avium* detected in eleven urine specimens (17.4%) and only two were recovered from sputum specimens (3.2%) under investigation.

(Table 2) Distribution of different enterococcal species by type of spscimens.

Clinical specimens	*Enterocoocus faecalis*		*Enterococcus faecium*		*Enterococcus avium*	
	No.	%	No.	%	No.	%
Urine	30	47.6	13	20.6	11	17.4
Wound	0	0	2	3.2	0	0
Throat swab	4	6.4	1	1.6	0	0
Sputum	0	0	0	0	2	3.2
Total	34	54	16	25.4	13	20.6

N.B. % were correlated to the total number of isolated enterococcal species (63).

Vancomycin resistance of the isolated enterococcal isolates to vancomycin was presented in (table 3). The results indicated that out of 34 *Enterocoocus faecalis* isolates; 28 (82.4%) were susceptible to vancomycin and 6 (17.6%) were resistant to vancomycin. Also, out of 13 *Enterocoocus avium* isolates; 12 (92.3%) were susceptible to vancomycin and only one (7.7%) was resistant to vancomycin. However, all isolated *Enterococcus faecium* were found to be susceptible to vancomycin.

Table (3) Susceptibility of different enterococcal species to vancomycin.

Enterococal species	Suscptibility to vancomycin				Total	
	Vancomycin sensitive		Vancomycin resistant			
	No.	%	No.	%	No.	%
Enterocoocus faecalis	28	82.4	6	17.6	34	100
Enterococcus faecium	16	100	0	0	16	100
Enterococcus avium	12	92.3	1	7.7	13	100

N.B. % were correlated to the total number of individual enterococcal species.

DISCUSSION

Enterococci are isolated from the clinical specimens and are gaining upper hand in the causation of nosocomial infection[7].

Enterococci are the second leading cause of nosocomial infection, joining *Escherichia coli*, *Pseudomonas aeruginosae* and *Staphylococcus aureus* in the list of most prevalent pathogens[8].

When clinical isolates of vancomycin resistant enterococci (VRE) began to appear in the late 1980s, it prompted significant changes in testing of enterococci in the clinical microbiology laboratory, infection control of enterococci and treatment of enterococcal infections[9].

There is lack of data about the prevalence of VRE in the Libyan hospitals and one of the major goals of this study was to evaluate prevalence of VRE in clinical specimens obtained from different patients attending Al-khumes Teaching Hospital, at Al-khumes City, Libya.

In the current study, the incidence rate of isolation of enterococcal species from the examined clinical specimens was 31.5% which is quite different from that obtained by Al-Jarousha *et al* (2008), they found that the incidence of enterococcal infection was 1.9%[10]. Also a different incidence rate (5.9%) was observed in another study **[11].** The prevalence rate of enterococci infection recorded in this study is considered high compared with other studies in the same field. In view of the fact that all the isolates were from clinically infected patients over a period of more than one year. However, a study in India provided similar results to the current study; enterococci were found in 22.2% of the clinical specimens,

with Foley catheters and burn wounds being the major sites of isolation[12].

In the present study, specimens obtained from urinary tract infected patients were the most common source of enterococci. 38.3% of urine specimens collected were positive for enterococci; followed by 33.3 % from sputum specimens, 13.5 of wound infections, and 12.5% from throat swab specimens. This was more or less in agreement with Moldering, who reported that enterococci implicated in 35% of nosocomial urinary tract infected patients[13]. In Kuwait hospitals reported that UTIS are the most nosocomial infections 36.6% caused by enterococci then it was prevalent in 11% wound swabs[14]. In addition, a similar results obtained by Salem (2005), who reported that enterococci prevalent in 34.02% urinary tract infections, 30.16% of blood specimens 30.16% of stool specimens, 26.83% of burn infection, 22.86% of wound infections, and 21.05% of ascetic fluids **[15].** However; the results of the current study were not in agreement with records obtained by other authors. Desai *et.al.*(2001) reported that enterococci were involved in 29.5% of burn infections and was detected in 21% of surgical wound infection[12]. Also in India Karmarkar *et. al.,(2004)* reported that enterococci were responsible for 10.27% of hospital acquired urinary tract infections[16].

In the present study, the most isolated Enterococcal species 54% were, Enterococcus faecalis, 25.4% Enterococcus faecium and 20.6% Enterococcus avium. In agreement with the current study, Enterococcus faecalis and Enterococcus faecium are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Other enterococcal species known to cause human infection include Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus and Enterococcus mundtii[17].

In Egypt, Salem et al., (2001), reported that the majority of the isolated Enterococcus spp. in clinical isolates were 84.5 % Enterococcus faecalis follwed in a descending order by 8.3% Enterococcus faecium, 2.08% Enterococcus avium, 1.04% Enterococcus durans and 1.04% Enterococcus gallinarum[15].

In a study in Lebanon,153 consecutive clinical enterococcal isolates collected between 1998 and 1999 were identified by conventional methods and API-Strep System were found to be 72.6% Enterococcus faecalis 22.9% Enterococcus faecium 3.2% Enterococcus avium and 1.3% Enterococcus gallinarum. None of the isolated showed resistance to vancomycin, except for one Enterococcus gallinarum isolate[18].

In a study in Kuwait hospitals, from 415 isolates 85.3% Enterococcus faecalis, 7.7% Enterococcus faecium, 4% Enterococcus casseliflavus, 1.2% Enterococcus avium, 1% Enterococcus durans, 0.5% Enterococcus gallinarum and 0.2% Enterococcus bovis. All were tested against vancomycin using disc diffusion method. They were resistant to vancomycin (2.6%)[14].

Vancomycin-resistant *Enterococcus* (VRE) has increasingly been implicated as a causative pathogen in nosocomial infections[19]. Susceptibility testing of different enterococcal species isolated in this study revealed that out of 38 Enterocoocus faecalis isolates; 28 (82.4%) were susceptible to vancomycin and 6 (17.6%) were resistant to vancomycin. Also, out of 13 Enterocoocus avium isolates; 12 (92.3%) were susceptible to vancomycin and only one (7.7%) was resistant to vancomycin. However, all isolated Enterococcus faecium were found to be susceptible to vancomycin. A different findings reported by some other authors; they found that Enterococcus faecium strains were resistant to vancomycin most often through the action of resistance operons encoding Van A and Van B types resistance[20].

CONCLUSION AND RECOMMENDATION

Enterococcal isolates from the throat swab were more or less equally distributed among both male and female patients. Isolates from urine specimens show higher in female patients than the male. Hospital personnel should be screened for antibiotic resistance, necessary steps to be taken to control of antibiotic use in hospitals and shortening the duration of hospital stay may help to control the spread of vancomycin resistant enterococci.

REFERENCES

1- Koneman, E. W.; Allen, S. D., Janda, W. M., Schreckenberger , P. C. and Winn, W. C. jr. eds. (1997): Color atlas and textbook of diagnostic microbiology, 5 th. Ed., Lippincot-Raven publishers, Philadelphia, USA.

2- Shaikh , *Z. H. A.*; Peloquin, C. A. and Ericsson, C. D. (2001): Successful treatment of vancomycin-resistant Enterococcus faecium meningitis with Linezolid: case report and literature review. *Scand. J. Infec. Dis.* 33: 375-379.

3- Mazuski J. E. (2008): Vancomycin-resistant enterococcus: risk factors, surveillance, infections and treatment. *Surg Infect 9*: 567-571.

4- Werner G., Coque T. M., Hammerum A. M., Hope R., Hryniewicz W., Johnson A., Klare I., Kristinsson K. G., Leclercq R., Lester C. H., Lillie M., Novais C., Olsson-Liljequist B., Peixe L. V., Sadowy E., Simonsen G. .S, Top J., Vuopio-Varkila J., Willems R. J., Witte W. and Woodford N. (2008): Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill 13*: 1-11.

5- Kaye K. S., Engemann J. J., Fraimow H. S., Abrutyn E. (2004): Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanisms, and clinical management. *Infect Dis North Am.* 18:467-511.

6- CLSI - Clinical Laboratory and Standards Institute (2009): Perfor-mance standards for antimicrobi-

al susceptibility testing. Nineteenth Informational Supplement M100-S19, *CLSI*, Wayne, pp. 177.
7- Hallgren, A.; Abednazari, H.; Ekdahl, C.; Hanberger, H.; Nillson, M.; Samuelsson, A.; Nilsson, L. E. and the Swedish ICU Study Group (2001): Antimicrobial susceptibility patterns of enterococci in ICU in Sweden evaluated by different MIC breakpoint system. *J. Antimicrob. Chemother*. 48: 53-62.
8- Alexander A. Padiglione, Rory Wolfe Elizabeth A. and Grabsch. 2003: Risk Factors for New Detection of Vancomycin-Resistant Enterococci in Acute-Care Hospitals That Employ Strict Infection Control Procedures. *Antimicrob. Agents Chemother* 47(8): 2492-2498.
9- Huycke, M. M.; Sahm, D. F. and Gilmore, M. S. (1998): Multiple-drug resistant enterococci: The nature of the problem and an agenda for the future. *Emerg. Inf. Dis*. 172: 273-276.
10- Al-Jarousha A. M. J.; Saed A. M. and Afifi H. (2008): Prevalence of Multidrug Resistant Enterococci in Nosocomial Infection in Gaza Strip. *Al-Aqsa Unv. J*. 12.
11- Olawale, K. O; Fadiora, S. O.and Taiwo, S. S. (2011): Prevalence of hospital-acquired enterococcal infections in two primary care hospital in Osogbo, Southeastern Nigeria. *Afr. J. Infect. Dis. 5(2): 40 – 46.*
12- Desai P. J.; Pandit D; Mathur M. and Gogate A. (2001): Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. *Indian J. Med. Microbiol.* 19:132–7.
13- Moellering, R. C. (1992): Emergence of *Enterococcus* as a significant pathogen. *Clin. Inf. Dis.* 14: 1173-1178.
14- Udo, E. E.; Al-Sweih, N.; Phillips, O. A. and Chugh, T. D.(2003): Species prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals. *J. Med. Microbiol*. 52:163-168.
15- Salem, M. M. (2005): The presence of vancomycin resistant Enterococci in some Egyptian Hospitals. Thesis submitted for the degree of doctor of Pharmaceutical Sciences in Microbiology. Alazhar University, Cairo, Egypt.
16- Karmarkar, M. G.; Gershom, E. S. and Mehata, P. R. (2004): Enterococcal infections with special reference to phenotypic characterization and drug resistance. *Indian J. Med. Res*. 119:22-25.
17- de Perio, M. A.; Yarnold P. R. and Warren, J. (2006): Risk factors and outcomes associated with non-*Enterococcus faecalis*, non-*Enterococcus faecium* enterococcal bacteremia. *Infect Control Hosp Epidemiol*. 27(1): 28-33.
18- Zouain, M. G. and Araj, G. F. (2001): Antimicrobial resistance of enterococci in Lebanon. *Int. J. Antimicrob. Agents* 17: 209-213.
19- Iwen, P. C.; Kelly, D. M.; Linder, J. and Hinrichs, S. H. (1996): Revised approach for identification and detection of ampicillin and vancomycin resistance in *Enterococcus spp*. by using Micro Scan Panels, *J. Clin. Microbiol.* 34(7): 1779-1783.
20- Hanrahan, J.; Hoyen, C. and Rice, L. B. (2000): Geographic distribution of a large mobile element that transfers ampicillin and vancomycin resistance between *Enterococcus faecium* strains. *Antimicrob. Agents Chemother.* 44(5): 1349-1351.