Research Paper

Healthcare-associated vancomycin resistant *Enterococcus faecium* infections in the Mansoura University Hospitals intensive care units, Egypt

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Abstract

Vancomycin resistant *Enterococcus faecium* (VREF) ia an emerging and challenging nosocomial pathogen. This study aimed to determine the prevalence, risk factors and clonal relationships between different VREF isolates in the intensive care units (ICUs) of the university hospitals in our geographic location. This prospective study was conducted from July, 2012 until September, 2013 on 781 patients who were admitted to the ICUs of the Mansoura University Hospitals (MUHs), and fulfilled the healthcare-associated infection (HAI) criteria. Susceptibility testing was determined using the disk diffusion method. The clonal relationships were evaluated with pulsed field gel electrophoresis (PFGE). Out of 52 *E. faecium* isolates, 12 (23.1%) were vancomycin resistant. The significant risk factors for the VREF infections were: transfer to the ICU from a ward, renal failure, an extended ICU stay and use of third-generation cephalosporins, gentamicin, or ciprofloxacin. PFGE with the 12 isolates showed 9 different patterns; 3 belonged to the same pulsotype and another 2 carried a second pulsotypes. The similar pulsotypes isolates were isolated from ICUs of one hospital (EICUs); however, all of the isolates from the other ICUs had different patterns. Infection control policy, in conjunction with antibiotic stewardship, is important to combat VREF transmission in these high-risk patients.

Key words: *Enterococcus faecium*, ICU, vancomycin, risk factors, PFGE.

Introduction

Enterococci are opportunistic pathogens of the normal humans and animals intestinal microbiota. The most common *Enterococcus* species that is involved in nosocomial infections is *Enterococcus faecium* (*E. faecium*) (Top *et al.*, 2008; Arias and Murray, 2012).

E. faecium has become one of the most important, emerging and challenging nosocomial pathogens (Arias and Murray, 2012). It is a difficult to treat this pathogen due to its intrinsic resistance to cephalosporins, aminoglycosides, clindamycin and trimethoprim-sulfamethoxazole (Leclercq *et al.*, 2013). Moreover, it has the ability to easily acquire antibiotic resistance genes trough transfer of plasmids, chromosomal exchange or mutation (Jett *et al.*, 1994).

In addition to this distinct resistance profile, genomic analyses have shown that hospital acquired *E. faecium* strains have a genetic repertoire that is distinct from that of community associated *E. faecium* strains that asymptomatically colonize the human gastrointestinal tract (van Schaik *et al.*, 2010). This distinct genetic repertoire includes the enterococcal surface protein, Esp, which is a known virulence determinant (Heikens *et al.*, 2007; 2011). Genomic islands that encode novel metabolic pathways (Heikens *et al.*, 2008), and insertion sequence elements (Leavis *et al.*, 2007). It is now considered that these determinants may be adaptive elements that have improved the relative fitness of *E. faecium* subpopulations in the hospital environment (Willems and van Schaik, 2009). Due to the resistance of multiple antibiotics, the treatment of choice in serious *E. faecium* infections is glycopeptides. However, prudent use of vancomycin is needed as it is associated with

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an increased risk for vancomycin resistant *enterococci* (VRE) colonization and infection (Tornieporth *et al.*, 1996).

In the last decades, the number of VRE infections has increased (Grayson *et al.*, 1991; Jones *et al.*, 1995; Rice, 2001). The first VRE isolates were reported in the United Kingdom in the late 1980s (Uttley *et al.*, 1988). In the United States, more than 80% of *E. faecium* isolates from hospitals are vancomycin resistant (Hidron *et al.*, 2008). Vancomycin-resistant *E. faecium* (VREF) has been associated with outbreaks in hospitals worldwide (Arias and Murray, 2012). The VREF colonization and infection rates have risen steadily, with most cases being caused by the VanA and VanB genotypes, which are the most commonly encountered glycopeptide resistance forms (Coque *et al.*, 1996; Rice, 2006; Deshpande *et al.*, 2007).

The clinical impacts of VRE include the limited availability of drugs to treat VRE infections and the ability of VRE to transfer the genetic determinant for vancomycin resistance to other Gram-positive pathogens, such as *Staphylococcus aureus* (Schooneveld *et al.*, 2008; Noble *et al.*, 1992).

VREF is associated with hospital-acquired infections such as urinary tract infections, wounds, bacteremia, endocarditis and meningitis (Top *et al.*, 2008; Arias and Murray, 2012). Several studies have demonstrated that VRE bacteraemia patients have a higher mortality rate than those infected with vancomycin-susceptible *enterococci* (Vergis *et al.*, 2001; Edmond *et al.*, 1996; Bhavnani *et al.*, 2000).

Information regarding the VRE Infections prevalence in Egypt indicates that there is an increasing VRE infection rate. Ghonaim *et al.* (2009) reported that VRE isolates constituted 20.9% of hospital associated enterococcal infections at the Egyptian National Liver Institute.

To control the rapid spread of multidrug resistant organisms, it is necessary to understand the risk factors for acquiring them. Therefore, the aim of this study was to identify the VREF prevalence and possible risk factors in ICU patients who are at high risk of VREF infection. Additionally, we aimed to identify the clonal relationship between different isolates via pulsed-field gel electrophoresis (PFGE).

Materials and Methods

Patients and methods

Study design

This was a prospective study, which was conducted in nine ICUs in three different MUHs, that were situated within the same geographical region over a 15 months period from July, 2012 until September, 2013. The ICU bed numbers range from 4 to 27, with a median of 10. They ICUs were categorized as: A. emergency hospital ICUs (EICUs), B. specialized medical hospital ICUs (SMICUs)

and C. children hospital ICUs, which included pediatric (PICU), neonatal ICU (NICU) and surgical ICU (SICU).

Study population

This study included 781 patients (who provided written consent) that were admitted to different MUHs ICUs and fulfilled the healthcare-associated infection (HAI) criteria. (Infections acquired ≥ 48 hours after admission that were not present or incubating at the time of hospital admission). The infection types were documented with specific HAIs definitions, which were established by the Centers for Disease Control and Prevention (Horan *et al.*, 2008).

Data collection and definitions

The following data were collected: demographic characteristics, ICU stay duration, transfer to the ICU from a ward, diagnosis at ICU admission, medical conditions that may alter the patient's immunity (comorbidity) (*e.g.* diabetes, malignancy, hepatic impairment, renal impairment and chronic lung diseases), invasive devices or procedures (*e.g.* surgical procedures, central venous catheter [CVC], peripheral venous catheter [PVC], mechanical ventilation, and total parenteral nutrition [TPN], drug therapy (*e.g.* antibiotics and their durations, prior antibiotic treatment, which was defined as any antibiotic treatment during the two weeks preceding ICU admission), immunosuppressive therapy (which included steroid therapy, cytotoxic chemotherapy or radiotherapy given within one month prior to ICU admission), and neutropenia (less than 500 neutrophils per mm^3)

A "case patient" was defined as patient who was infected with VREF and was attending a Mansoura University ICU during the study period. A "control patient" was defined as a Vancomycin susceptible *Enterococcus faecium* (VSEF) infected patient who was attending a Mansoura University ICU during the same period. The patients whose initial isolate was susceptible to vancomycin but who subsequently had VREF isolates that were recovered were included as case patients.

Microbiologic studies

The samples were processed in the Medical Diagnostics and Infection Control Unit (MDICU) laboratory using standard protocols. *E. faecium* laboratory identification was performed by Gram stain, colonial morphology on blood agar, growth and blackening of bile esculin agar, the absence of catalase production, resistance to 6.5% sodium chloride. The results were confirmed with the API 20 Strep (Biomerieux SA, Montalieu - Vercica and France) system. In vitro susceptibilities of the isolates to antimicrobials were determined with the disk diffusion method which was defined by the [CLSI] (Institute CaLS, 2011). The antibiotics that were tested included ampicillin (10 μg), ciprofloxacin (5 μg), high content gentamicin (120 μg), erythromycin (15 μg), tetracycline (30 μg), vancomycin (30 μg),

teicoplanin (30 μg), linezolid (30 μg), and chloramphenicol (30 μg). Glycopeptide antibiotics MICs for: vancomycin and teicoplanin against the *E. faecium* isolates were examined with the VITEK 2 system (bioMérieux).

PFGE

The clonal relationships between the vancomycin-resistant strains were studied by evaluating the genomic DNA with PFGE (Antonishyn *et al.*, 2000). DNA that was restricted with the SmaI enzyme was separated on an agarose gel using a CHEF DR III apparatus (Bio-Rad laboratories). The running conditions were 6 V per cm, with pulses ranging from 2 to 15 s for 18 h at 14 μC. The DNA banding patterns were visualized under UV light after staining with ethidium bromide (0.5 mg/mL). The similarities between the isolates were determined by visual comparison of the isolate band patterns. The interpretation of the PFGE results was carried out by eye according to the criteria described by Tenover *et al.* (1995).

Statistical analysis

The SPSS software (SPSS Inc., Chicago, IL, USA) was used for data analyseis. Proportions were compared using the χ2 test and continuous variables were compared using Student's t or Mann-Whitney U tests. The results are presented as numbers (percentages) for frequency and as the mean ± standard deviation for quantitative variables. Odds ratios (OR) [95% confidence interval (CI)] were calculated for all significant (p < 0.05) qualitative variables in the univariate analyseis.

Results

VREF Prevalence in the ICUs

During the study period, a total of 975 specimens were collected from 782 patients, with clinically suspected HAIs in MUHs ICUs. Out of 52 *E. faecium* isolates detected, 12 were VREF, which constituted (12/52) 23.1% of the *E. faecium* isolates.

VREF isolates

Twelve VREF single-patient isolates (*i.e.*, 1 isolate per patient) were obtained from the following sites: urine (7 isolates), blood (3 isolates) and wound (2 isolates). A total of 12 infections were documented in 12 patients, including urinary tract infection (7 patients), bacteremia instances (3 patients), and surgical site infection (2 patients). Of these 12 patients, 6 isolates were obtained from the emergency hospital ICUs, 4 were obtained from the ICUs of the specialized medical hospital and 2 were from PICUs.

Antibiotic susceptibility testing of the VREF isolates

Twelve *E. faecium* isolates were resistant to both glycopeptides antibiotics (vancomycin and teicoplanin) (with a vancomycin MIC ≥ 32 μg/mL) (VanA phenotype).

All VREF isolates (100%) were resistant to ampicillin, gentamicin, and ciprofloxacin. Most of the isolates (92%) were resistant to erythromycin (only one isolate was sensitive), and 5 isolates were resistant to tetracycline (42%). All of the strains were sensitive to linezolid and chloramphenicol.

The ICU-acquired VREF Risk factors

Among the 52 *E. faecium* infected patients, 40 (76.9%) had VSEF infections (control patients), and 12 (23.1%) had VREF infections (case patients). The comparative demographic and clinical features for both the case and controls patients are listed in Tables 1 and 2. The risk factors that were significantly associated with the ICU-acquired VREF infections were: transfer to the ICU from a ward (p = 0.04), renal failure (p = 0.01), and longer ICU stay duration (p = 0.018). Additionally, antibiotic use for a long period of time and use of third-generation cephalosporins, gentamicin, or ciprofloxacin were also associated with VREF infections.

PFGE

The restriction endonuclease patterns obtained with the PFGE following the SmaI treatment for the 12 VREF isolates are presented in Figure 1. In general, the 12 isolates showed 9 different patterns. Three isolates belonged to the same pulsotype and another 2 carried similar pulsotypes. Of interest, the isolates with similar pulsotypes were isolated from the ICUs of one hospital (EICUs); however, all of the isolates from the other ICUs had different patterns.

Discussion

E. faecium is a highly resistant nosocomial pathogen and has recently emerged as an important threat in hospitals worldwide (Arias and Murray, 2012). In this study, VREF constituted 23.1% of the observed *E. faecium* isolates, a result that was close to the Ghonaim *et al.* study observations, in which the VRE isolates constituted 20.9% of the hospital-associated enterococcal infections at the National Liver Institute (Ghonaim *et al.*, 2009), which indicated a big problem in Egypt. Similar studies from the United States reported vancomycin resistance in up to 28% of all nosocomial *Enterococcus* species (NNIS, 2004). However studies from India and South America showed much lower of VRE infection prevalence ranging from 1- 8.7% (Mathur *et al.*, 2003; Taneja *et al.*, 2004; Kapoor *et al.*, 2005; Kaur *et al.*, 2009; Praharaj *et al.*, 2003; Panesso *et al.*, 2013). *E. faecium* antibiotics sensitivity pattern in the present study is consistent with the concept that vancomycin resistance is usually accompanied by resistance to other antimicrobial agents, such as penicillin, ampicillin, erythromycin, ciprofloxacin and gentamicin. The objective of this study was to determine the risk factors for HAI that were associated with VREF among the MUH ICU inpatients. We found that transfers to the ICU from a ward (p = 0.04) and renal fail-

Table 1 - ICU-acquired VREF infection risk factors.

Factor	Patients with *E. faecium* infections				p- value	Odds ratio
	VREF n = 12		VSEF n = 40			
	No.	(%)	No.	(%)		
Sex (male):	7	58.3	25	62.5	0.8	0.84
Diabetes mellitus	3	25.0	7	17.5	0.7	1.6
Neutropenia	4	33.3	10	25.0	0.7	1.5
Neoplastic disease	5	41.7	16	40.0	0.9	1.1
Transfer to the ICU from a ward	8	66.6	12	30.0	**0.04***	4.7
Surgery	4	33.3	14	35.0	1	0.9
Immunosuppressive drugs	5	41.7	11	27.5	0.4	1.9
Prior antibiotic treatment	10	83.3	28	70.0	0.5	2.1
Organ failure:						
Respiratory	3	25	6	15	0.4	1.9
Cardiac	2	16.7	7	17.5	1	0.9
Renal	5	41.7	3	7.5	**0.01***	8.8
Neurologic	1	8.3	6	15.0	1	0.5
Liver	3	25	5	12.5	0.4	2.3
During ICU hospitalization:						
Intravascular catheter	12	100	36	90	0.6	-
Urinary catheter	12	100	37	92.5	1	-
Mechanical ventilation	12	100	37	92.5	1	-
ICU stay duration (days)	12 ± 5.51		9.23 ± 4.86		**0.018***	0.47-5.06

* Significant, p-value ≤ 0.05; ICU, intensive care unit.

Table 2 - Antibiotic use amongst the study patients during their ICU stays.

Factor	Patients with *E. faecium* infections				p-value	Odds ratio
	VREF n = 12		VSEF n = 40			
	No.	(%)	No.	(%)		
Antibiotic treatment duration, days	16 ± 11		5 ± 6		**< 0.001****	8.09-13.91
Vancomycin use	5	41.7	12	30.0	0.45	1.66
Extended-spectrum penicillin use	7	58.3	19	47.5	0.5	1.6
Fluoroquinolone use	9	75	16	40.0	1	0.8
Carbapenem use	3	25.0	12	30.0	1	0.8
Aminoglycoside use	6	50.0	8	20.0	**0.04***	4
First generation cephalosporin use	2	16.7	18	45.0	0.09	0.2
Second generation cephalosporin use	2	16.7	15	37.5	0.3	0.3
Third generation cephalosporin use	6	50.0	6	15.0	**0.01***	5.7

*Significant, p-value ≤ 0.05; **highly significant, p-value ≤ 0.001.

ures (P = 0.01) were associated with VREF infections. Intra hospital transfer was associated with VREF colonization or infection in other studies (Tornieporth *et al.*, 1996; Webb *et al.*, 2001). The patients may have acquired *E. facium* during their ward stay, which later caused infections during their ICU stays. However, our study showed that renal failure was a risk factor for VREF infection acquisition. This is different from other studies in which dialysis was not associated with the VREF infection incidences (Webb *et al.*, 2001; Handwerger *et al.*, 1993; Descheemaeker *et al.*, 2000). Patients who require hemodialysis often have complicated illnesses and may receive multiple antibiotic courses, including vancomycin, which places them at greater risk for VREF infection or colonization. Frequent hospital-
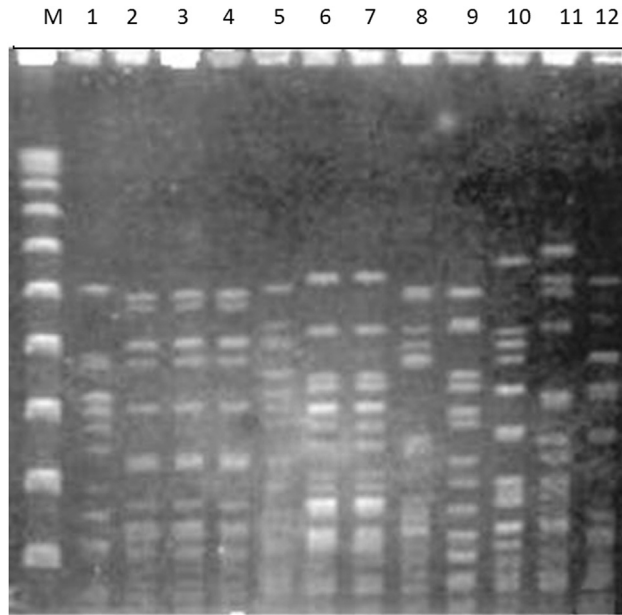
Figure 1 - SmaI restriction endonuclease patterns obtained by PFGE: Lane M marker; lane 1, patient 1 (SMICU); lanes 2, 3and 4 (EICUs); lane5, (PICU); lanes 6 and 7, (EICUs); lane 8, (PICU); lane 9, (SMICU); lane 10, (SMICU); lane 11, (SMICU); lane 12, (EICU).

izations and cross-transmission can also contribute to VREF infection in patients undergoing hemodialysis (Chow *et al.*, 1993; Roghmann *et al.*, 1998). The other risk factor for VREF infection in the ICU was longer ICU stay duration (p = 0.018). This result consistant with most previous studies (Ostrowsky *et al.*, 1999; Warren *et al.*, 2003; Se *et al.*, 2009; Pan *et al.*, 2012; Fridkin *et al.*, 2001). Longer ICU stays can indicate a greater chance of receiving antibiotics and also a longer exposure time to possible pathogen transmission. Our study showed that patients who had a prior exposure to broad-spectrum antibiotics and for a prolonged duration were at a higher risk of VREF infections due to the selective pressure from prior antibiotics. Interestingly, in this study, vancomycin use was not found to be a risk factor for VREF infection, which is different from other studies (Tornieporth *et al.*, 1996; Fridkin *et al.*, 2001). This can be explained by the theory that in medical settings in which VREF infections are endemic, the patients with VREF colonization may serve as a source for already antibiotic resistant *E. faecium* strains in patients who have not necessarily received glycopeptide antibiotics (Murray, 2000; Hayden, 2000). According to the PFGE analysis, there were nine pulsotypes that were noted during the study period. The PFGE analysis showed different patterns in the different ICUs of the three hospitals, except for the EICUs in the emergency hospital, in which the 6 VREF isolates showed 3 different patterns. Specifically, 3 isolates belonged to the same pulsotype and another 2 carried similar pulsotypes, indicating that there was a spread within the ICUs of one hospital (the emergency hospital). Thus, our

data show that all of the strains were not from the same clone, indicating multiple acquisitions of resistant isolates. However, the VREF infections within the ICUs of one hospital (the EICUs) may result from cross transmission of prevalent isolates. This shows that spread occurred within the ICUs of an individual hospital but not in the other hospitals. This might be because the EICUs have more critical care cases compared with the other ICUs (*e.g.*, care for patients with acute, life-threatening illnesses or injuries), in which VRE infection prevention measures are more important than in the other ICU type's. Similarly other studies have documented the spread of VREF clones among hospitals (Sader *et al.*, 1994; Fridkin *et al.*, 1998; Nourse *et al.*, 2000; Corso *et al.*, 2007). The microorganism can be transmitted by health care workers in particular via their hands which are most likely the most common mode of nosocomial transmission (Boyce *et al.*, 1994). VREF transmission by way of contaminated medical equipment and health care carriers has been investigated and shown to occur in different hospitals sections especially in the dialysis ward (Kalocheretis *et al.*, 2004). Adherence to infection control precautions by ICU staff members may also affect possible VREF transmission. Other distinct strains may come from intrinsic *E. facium*, which is selected for under selective pressure. Thus, infection control policy, in conjunction with practices that control antimicrobial use, is important to combat VREF infection transmission in these high-risk patients. Moreover, our data ensure that standard efforts to reduce cross-transmission might be needed hospital-wide because the VREF rates outside of the ICUs greatly affect the ICU- specific VREF rates.

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