Prevalence of Multidrug Resistant Enterococci in Nosocomial Infection In Gaza Strip

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الملخص

مدى انتشار الميكروب السبحى المعدي (Enterococci) متعدد المقاومة للمضادات الحيوية في عدوى المستشفيات في قطاع غزة

ميكروب السبحى المعدي (Enterococci) المسبب لعدوى المستشفيات مثـل التهـاب التسمم الدموي، و التهاب جروح العمليات، و التهاب الجهاز التنفسي السفلي، والتهاب المجـاري البولية. هذه الميكروبات أصبحت مقاومة للعديد من المضادات الحيوية و خصوصاً المـستخدمة بصورة روتينية. معظم أنواع العدوى كان سببها ميكروب ال Enterococcus fecalis والتـي أظهرت أن لها قدرة مرضية و ضراوة أشد في إحداث العدوى. أما بقية الإصابات فكان سـببها ميكروب ال Entreococcus faecium و هو معروف أنه ضعيف و لكنه ظهر أنه يقاوم العديد من المضادات الحيوية. تم فحص 309 عينات مرضية شاملة (116 عينه بـول، 104 عينـات صديد، 67 عينة دم، و 22 عينة بلغم) تم عمل التحاليل البكتيريولوجية اللازمة لها للكشف عـن مدى انتشار هذه الميكروبات في عدوى المستشفيات. الدراسة أظهرت أن الإصـابة بـالميكروب السبحي المعدي هو 1.9% من مجمل عدوى المستشفيات. المضادات الحيوية الفعالة كانت كمـا يلي:

بينمـا Linzolid, Nitrofurantoin, Chloramphenicol, and Ciprofloxacin, المضادات Amikacin, Gentamycin, Erythromycin and Lincomycin أظهرت تأثير معتدل.

أما المضادات التالية: وهي Methicillin, Ampicillin, Tetracycline, Cefuroxime and Cefotaxime فقد أظهرت النتائج أن الميكروبات كانت مقاومة لها. وظهرت أن هناك سـلالات مقاومة للمضاد Vancomycin .

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كذلك لا يوجد أي من مـضادات الCephalosprines مثـل: المـضادات Monobactams, Aztreonam or Semisynthitic penillins (Naficillin, Oxacillin) أي تأثير على هـذه الميكروبات. توزيع حالات الاصابه بيمكروب السبحي المعدي أظهرت نسبة عالية في مرضـى التهاب الجهاز التنفسي السفلى بواقع 4.5%، يليه التسمم الدموي 3%، يليه التهابـات الجـروح المقيحة 1.9%، و أخيراً التهابات المجاري البولية 0.8%

الكلمات المفتاحية: ميكروب السبحي المعدي، عدوى المستشفيات، تعدد المقاومة للمضادات الحيوية.

Abstract

Enterococci leading causes of nosocomial infection like bacteremia, surgical wound infection, lower respiratory tract, and urinary tract infection, these organisms are becoming resistant to many and sometimes all standard antibiotics, most enterococcal infections are caused by *Enterococcus faecalis* which are more likely to express traits related to over virulence. The remaining infections are mostly caused by *Enterococcus faecium*, a species virtually devoid of known overt pathogenic traits but more likely to be resistant to even antibiotics. 309 clinical samples included (116 Urine, 104 Pus, 67 Blood, and 22 Sputum) samples were processed to find the prevalence rate of enterococci and identify the species of clinical isolates of enterococci. Screening of various clinical specimens revealed that enterococci were prevalent in 1.9% of the total nosocomial infected cases. The most effective antibiotics were Linzolid followed by Nitrofurantoin, Chloramphenicol and Ciprofloxacin. Where Amikacin, Gentamicin, Erythromycin and Lincomycin showed moderate activities. At the same time Methicillin, Ampicillin, Tetracycline, Aztreonam, Cefuroxime and Cefotaxim showed resistance. Non of the available Cephalosporins, Monobactams, Aztreonam or semisynthetic Penicillins (Naficillin, Oxacillin) have any activity against enterococci. Vancomycin resistant strains were recorded in this study The distribution of enterococci cases showed highest rate in lower respiratory tract infection (4.5%), blood stream infection (3%), surgical wound infection (1.9%), and urinary tract infection (0.8%).

Key wards: Enterococci, Nosocomial infection, Multidrug resistance

Introduction:

Enterococci are constitutive members of the intestinal flora of humans and animals, and may also colonize the upper respiratory tract, biliary tracts and vagina of otherwise healthy persons **(Gin and Rzhanel, 1996, and Murray, 2000)**. Enterococci have been documented to cause infection of the urinary tract and other sites **(Wong, *et al* 2000)**. Enterococci account for approximately 110.000 urinary tract infections, 25000 cases of bacteremia, 40.000 wound infections and 1100 cases of endocarditis annually in the United States **(Haley, *et al* 1985, Harris, 1992, and Emori and Gaynes, 1993).**

Although more than one dozen species of enterococci have been identified, *Enterococcus fecalis* and *Enterococcus faecium* account for approximately 85-90%and 5-10% of human enterococcal infections respectively**(Gin and Rzhanel, 1996, and Murray, 2000)**. Other enterococcus species, *E. gallinarum, E. casseiifaras, E durans, E. avium,* and *E. raffinoss* are isolated much less frequently and account for less than 5% of clinical isolates **(Wong, *et al* 2000, and Moellering, 1992)**.

Over the past decade, many if not most hospitals have found the epidemic increase in vancomycin resistant enterococci isolates cultured from hospitalized patients causing endocarditis, urinary tract infection, blood stream infection and wound infection **(Moellering, 1998)**. A 9 years study from United Kingdom determined the Vancomycin resistance, *Enterococcus faecium* isolated in blood cultures reached 6.3% in 1993, 20% in 1995, and 24% in 1998 **(Reacher, *et al* 2000)**. According to the Centers for Disease Control and Prevention (CDC), the percentage of enterococcal isolates that were resistant to Vancomycin reported by U. S intensive care units (ICU) increased from 0.3% in 1989 to 25.2% in 1999 **(CDC, 1999)**. The proportion of nosocomial enterococcal isolates in the United States that were resistant to Vancomycin 17% in 1999 was much higher than the proportion of Vancomycin resistant enterococcus isolated from patients in the rest of the world **(Low, *et al* 2001)**. Vancomycin resistant enterococci (VRE) colonization and infection occurs predominantly in patients with severe underlying disease, extended length of hospital stay, and previous antibiotic exposure, the most consistently recognized antibiotic agents including or facilitating the acquisition of VRE colonization, cephalosporins and anti-anaerobic agents **(Murray, 2000)**. Moreover the total volume of antibiotic agents and the duration of antibiotic treatment or prophylaxis seem to be important risk factors for the acquisition of VRE **(Harbarth, *et***

***al* 2000, Tokar, *et al* 1999, and Fridkin, *et al* 2001)**. The main reservoir for enteroccci in humans is the gastrointestinal tract, importantly if patients are colonized with very low numbers of VRE that are not detected by rectal swabs, emergence of these strains when patients are exposed to antibiotics might be incorrectly interpreted as true acquisition **(Sloughter, *et al* 1996)**. Therefore it is suggested that Vancomycin exposure may exert selective pressure on the agent raising undetectable levels of pre-existing VRE to detectable levels **(Fuller, *et a.*, 1998)**.

Materials and Methods

Various clinical specimens such as urine samples, infected wound samples, sputum, and blood stream samples were obtained in the microbiological department and processed by standard methods for the isolation of enterococci from European Gaza Hospital, during a period of January 2003 to December 2003. The specimens were plated on Pfizer's enterococcus selective agar which is a bile esculin medium for the isolation of enterococci, these specimens were also plated on blood agar and macConkey agar for the isolation of concomitant organism, along with enterococci **(Baily and Scott, 1994)**. Enterocci were identified on the basis of growth on bile esculin medium, Gram staining i.e., Gram positive cocci in pairs and short chains catalase negative, growth in 6.5% NaCl and at pH 9.6 **(Baily and Scott, 1994)**. Other tests such as Bacetracin resistance, fermentation of ribose and positive voges proskauer test. Enterococcal strains were further identified to the species level by using conventional physiological tests devised by Facklam and Collins **(Facklam and Collins, 1989)** which are based on carbohydrate fermentation using 1% solution of the following sugars: glucose, mannitol, rabinose, raffinose, sorbitol, sucrose, lactose, trehalose, and inulin by pyruvate utilization in 1% pyruvate broth. Arginine decarboxylation in Moeller's decarboxylase broth, hippurate hydrolysis. Motility test, pigment production detected on tryptic soy agar (TSA), gelatin liquefaction, starch hydrolysis using 2% starch and polysaccharide hemolysin production was detected on blood agar using 5% human blood **(Facklam and Collins, 1989)**.

The antimicrobial susceptibility test was carried out by using diffusion technique. Mueller hinton media was inoculated with calibrated number of the isolated pathogen $5x10^5$ CFU/ml (0.5 McFarland Standard). Inhibition zones were measured according to standard methods with taking in the consideration the nature and effective spectrum of each antibiotic **(Miles and Amyes, 1996)**.

Results

Six isolates of enterococci were isolated from a total of 309 various clinical samples described as nosocomial infections. The incidence of enterococcal infection was 1.9%. distribution of enterococci cases according to the site of infection was presented in table 1, lower respiratory tract showed the highest rate (4.5%) of the total bacterial isolates followed by blood stream infection (3%), wound infection 1.9% and urinary tract infection 0.8%

Table 1. Distribution of enterococci cases by site of infection

Site of infection	No. of enterococci isolates	Total No. of isolated pathogens from patients	Percentage of enterococci to total No. of isolated pathogens
Urinary tract infection	1	116	0.8
Wound infection	2	104	1.9
Blood stream infection	2	67	3
Lower respiratory tact infection	1	22	4.5
Total	6	309	1.9

The frequency of isolated enterococci in comparison to total isolates was present in table 2. The overall enterococcal infection rate was 1.9% of the total nosocomial infection (6 out of 309). Gram positive (including Enterococci) was 28.2% of the total nosocomial infection, while Gram negative pathogens constituted 68.6%and yeast 3.2%.

Table (2) Frequency of isolated enterococci in comparison to other isolated pathogens

Type of isolates	No. of isolates	Percentage of isolates
Enterococci	6	1.9
Gram positive (including enterococci)	87	28.2
Gram negative isolates	212	68.6
Yeast and Candida albicans	10	3.2

The multidrug resistance was defined as resistance to 3 or more types of antibiotics. The frequency of nosocomial multidrug resistant bacteria are present in table (3)

In table (3) 66.6% of the total enterococci was considered as multidrug resistant, also enterococci strains isolated from different wards of the hospital were 100% resistant for Vancomycin. (Enterococci in urinary tract infection, blood stream infection, and wound infection showed 100%

resistant for routine panel antibiotics except amoxycellin, augmentin, ampicillin, Ciprofloxacin, chloramphenicol and gentamycin.

The other isolated pathogens in our study showed variations in the antibiotics resistant patterns. The highest Multidrug resistant pathogen was *Enterobacter spp*. 100% followed by alpha hemolytic streptococci 87.5%, Acinetobacter bumanni 83.3%, Klebsiella spp. 64.5%, E. coli 45.5%, Staph hemolyticus 44.4%, Pseudomonas aerogenosa 40.0%, Coagulase negative staphylococci 27.2%, Staph aureus 18.7%, and Beta hemolytic streptococci 9%

Table (3) Frequency of nosocomial multidrug resistant resistant enterococci in comparison to other multidrug resistant bacteria

Type of pathogens	Frequency of isolated pathogen	No. of multidrug resistant bacteria	Percentage of multidrug resistant
Enterococci	6	4	66.6
Enterobacter spp.	3	3	100
Alph hemolytic streptococci	8	7	87.5
Acinetobacter bumanri	6	5	83.3
Klebsiella spp.	62	40	64.5
E.coli	77	35	45.5
Staphylococcus hemolyticus	9	4	44.4
Pseudomonas aerogenosa	50	20	40
Coagulase negative staphylococci	22	6	27.2
Staph aureus	32	6	18.7
Beta hemolytic streptococci	11	1	9

Discussion:

Enterococci normally inhabit the bowel, they are found in the intestine of nearly all animals, and humans Enterococci are readily recovered outdoor's from vegetation and surface water problem because of contamination by animal excretion as untreated sewage **(Jett, *et al,* 1994)**. During the period of January 2003 to December 2003 of the present study, enterococci was isolated from urine samples, infected wound samples, sputum, and blood stream samples from a patients admitted in European Gaza Hospital wards with the incidence rate 1.9%. the prevalence of enterococci in lower respiratory tract was the highest 4.1% followed by blood stream infection 3.0%, wound infection 1.9% and urinary tract infection 0.8%. Enterococci detected as one of the isolates in the clinical specimens with polymicrobial etiology which are better established as

pathogens and are primary target of subsequent therapy. Enterococci by virtue of being non invasive organisms **(Moellering, 1992)**. Colonization and infection with multidrug resistant enterococci (MDR) are world wide recorded cases. In the present study the percentage of multidrug resistant enterococci infections caused by Vancomycin resistant enterococci (VRE) was 66.6%. It was reported that antibiotic treatment with agents providing VRE with a selective growth transmission advantage such as Vancomycin or third generation cephalosporins has been shown to increase the risk of VRE colonization **(Bonten, *et al* 1996, and Karanfil, *et al* 1992)**. The prevalence of enterococci in clinical specimens can thus be attributed to their ability to grow and survive due to selective pressure of antimicrobial agents therefore the susceptibility strains are indicating by treatment of different antibiotic and the multidrug resistant include Vancomycin resistant are survive and colonize the patient sites of infection. Also enterococci are exceedingly hardy the tolerate a wide variety of growth conditions including temperatures of 10^0C to 45^0C and hypotonic acidic or alkaline environments, sodium azide and concentrated bile salts which inhibit or kill most microorganisms are tolerated by enterococci and used as selective agents in agar based media. As facultative organisms, enterococi have ability to grow under reduced or oxygenated conditions, also enterococci are usually considered as strict fermenters because they lack a Krebs cycle and respiratory chain **(Willett, 1992)**. *E. faecalis* is an exception since exogenous hemin can be used to produce d, b and o type cytochromes **(Ritchey and Seeley 1974)**. In the present study enterococci showed great variation in the response to the antibiotics and also resistant to most of the commonly regular used antibiotics in hospitals. Penicillin group are of limited use in the treatment of enterocccal infection, few of the available cephalosporins have activity against enterococci. Indeed enteroccocal superinfection is a major complications of cephalosporin usage, although quinolone antibiotics may be sufficiently active to treat enterococcal strains. Enterococci are intrinsically resistant to many antibiotics unlike acquired resistant and virulence traits, which are usually transposons of plasmid encoded, intrinsic resistance is based on chromosomal genes. Enteroccoci often acquired antibiotic resistance through exchange of resistance encoding genes carried on conjugative transposons, pheromone responsive plasmids and other broad host range plasmids **(Rice, *et al* 1995)**. Among several phenotypes for Vancomycin resistant enterococci Van A (resistance to Vancomycin and teicoplanin) and Van B (resistance to Vancomycin alone) are most common **(Arthur and Courvalin, 1993)**.

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